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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 (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.”¹
22 Analytical and sensorial characterization of aging bouquet
23 has been described for premium aged red wines produced in
24 Bordeaux^{2,3} and other regions.⁴ 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.⁵ The volatile
27 compounds identified in a wine are diverse in chemical
28 families, concentration ranges, and sensory contributions.⁶
29 Those with exceedingly low odor detection thresholds
30 (ODTs) are often aroma-critical compounds because of their
31 greater sensory impacts.⁷ Volatile thiols are such aroma-active
32 odorants that have been actively attracting research interest⁸
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.⁹ 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.¹⁰
41 Certain thiols were particularly linked to the empyreumatic
42 notes in aged wines.^{11–13} 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.⁵ 46

47 From the perspective of analytical chemistry, qualitative and
48 quantitative analyses of thiols in wine is intrinsically
49 challenging⁵ because these highly reactive molecules are
50 mostly present at ultratrace quantities (~ng/L) in a complex
51 wine matrix.¹⁰ 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.¹⁰ 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,¹⁴ was achieved
63 using such an analytical approach due to the extraordinarily
64 sensitive human olfactory system employed in GC–MS/O
65 screening.¹⁰ 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.^{14,15} A recently
 70 proposed protocol described the identification of new thiols in
 71 red wine through oak-wood accelerated reductive treatment.¹⁶
 72 Another alternative approach to identify thiols in foods and
 73 beverages¹⁰ 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⁺) cartridges for selective thiol extraction was
 81 developed in our laboratory, with a particular focus on
 82 qualitative thiol screening.¹⁷ In reported demonstrative
 83 applications, Ag⁺ 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.¹⁷ 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^{11,12} 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 (Cu²⁺)
 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⁺ SPE protocol.¹⁷ 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⁺
 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 (CH₂Cl₂) 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-Grattenstaden, 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 ^a	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 ^b
W2	2007	Cabernet Sauvignon 55%, Merlot 35%, Cabernet Franc 5%	13.0	1500	Saint-Julien ^b
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 ^b

^aBlending percentage not specified. ^bClassified growth.

with empyreumatic 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⁺ SPE. The extraction of thiols from wine was conducted
 according to a recently described protocol.¹⁷ A large-volume SPE
 barrel was fitted onto a Ag⁺ 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 CH₂Cl₂ (5% v/v solvent to sample
 ratio) three times. The resulting organic phases were pooled. Ag⁺ SPE
 cartridge was preconditioned by 10 mL of CH₂Cl₂. Afterward, the
 collected organic extracts were loaded onto a Ag⁺ SPE cartridge,
 followed by three washes, using CH₂Cl₂ (10 mL), acetonitrile (20
 mL), and CH₂Cl₂ (10 mL) in sequence. Final elution was conducted
 by 5 mL of H₂O, 20 mL of freshly prepared L-cysteine solution (10 g/
 L in H₂O, sparged with N₂ before use), and 10 mL of CH₂Cl₂. The
 collected elutes (5 mL of H₂O, 20 mL of L-cysteine solution, and 10
 mL of CH₂Cl₂) were combined together and stirred for 15 min, and
 the organic phases were pooled, dried on anhydrous Na₂SO₄, and
 concentrated under a gentle stream of N₂. For method blanks¹⁸
 (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).¹⁹ 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⁺ SPE

172 protocol described above. The final extract (~50 μ 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 method¹⁷ 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 N₂ (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 ¹D column (30 m \times 0.25 mm
188 i.d., 0.25 μ m, DB-5MS or DB-35MS, Agilent J&W) with a flow rate of
189 1.5 mL/min; LTM ²D column (30 m \times 0.25 mm i.d., 0.25 μ 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 ²D 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 μ 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 C₈–C₂₀ *n*-alkanes (Sigma-Aldrich)
208 for conventional GC–MS/O and for the ¹D of MDGC–MS/O runs.
209 LRIs for the ²D of the MDGC–MS/O were calculated according to a
210 previously described procedure.²⁰ 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 \times GC–TOF/MS.** GC \times 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 μ 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 \times 0.25 mm i.d., 0.25 μ m film thickness (Agilent J&W) and
222 RTX-17Sil MS 1.3 m \times 0.25 mm i.d., 0.25 μ m film thickness (Restek,
223 Bellefonte, PA), were used for the ¹D and ²D 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 (C₈–C₂₀, 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 Δ LRI \leq 30 and/or a
241 minimum mass spectrum similarity score (MSSS) of 800 out of 1000. 242

243 **2.7. Preparation of 1a and 1b.** The *trans*- and *cis*- stereoisomers
244 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

248 **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 F₂₅₄ 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 255
in 3.5 h. The mixture was extracted with EtOAc (3 \times 40 mL). The 256
organic phases were pooled and washed by H₂O (3 \times 40 mL), 257
neutralized with saturated NaHCO₃ solution (120 mL), and washed 258
by H₂O (2 \times 120 mL) until pH to 7. The organic phase was 259
separated, dried over anhydrous magnesium sulfate, and filtered. The 260
filtrate was collected, and the solvent was removed under a vacuum to 261
obtain a crude colorless liquid **2** (5.95 g). 262

263 **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/Et₂O, 1/9, v/v) to give two colorless liquids: **2a** (330 mg, R_f 265
= 0.28) and **2b** 1.195 g (R_f = 0.20). 266

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

269 *trans*-(±)-3-S-Benzoyl-2,5-anhydro-1,4-dideoxy-3-thiopentitol (**2a**): 269
¹H NMR (300 MHz, CDCl₃): δ 1.35 (d, *J* = 6.1 Hz, 3H, CH₃), 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_{ar}3',5'), 7.58 (tt, *J* = 7.4, 1.3 275
Hz, 1H, H_{ar}4'), 7.95 (dd, *J* = 8.4, 1.3 Hz, 2H, H_{ar}2',6'), ¹³C NMR (75 276
MHz, CDCl₃): δ 19.54 (CH₃), 33.63 (C4), 46.88 (C3), 66.98 (C5), 277
80.32 (C2), 127.38 (C_{ar}2',6'), 128.80 (C_{ar}3',5'), 133.66 (C_{ar}4'), 278
136.98 (C1'), 191.59 (C=O). 279

280 *cis*-(±)-3-S-Benzoyl-2,5-anhydro-1,4-dideoxy-3-thiopentitol (**2b**): 280
¹H NMR (300 MHz, CDCl₃): δ 1.27 (d, *J* = 6.0 Hz, 3H, CH₃), 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_{ar}3',5'), 7.58 (tt, *J* = 7.5, 1.3 Hz, 1H, H_{ar}4'), 7.95 (dd, *J* 287
= 8.4, 1.4 Hz, 2H, H_{ar}2',6'). ¹³C NMR (75 MHz, CDCl₃): δ 17.13 288
(CH₃), 33.84 (C4), 46.53 (C3), 66.25 (C5), 76.92 (C2), 127.38 289
(C_{ar}2',6'), 128.77 (C_{ar}3',5'), 133.60 (C_{ar}4'), 137.08 (C-1'), 191.65 290
(C=O). 291

292 **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 \times 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 CH₂Cl₂ (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

304 *trans*-(±)-2-Methyltetrahydrofuran-3-thiol (*trans*-**1a**): ¹H NMR 304
(300 MHz, CDCl₃): δ 1.29 (d, *J* = 6.1 Hz, 3H, CH₃), 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}C NMR (75 MHz, CDCl_3): δ 18.20 (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, 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**): ^1H NMR (300
323 MHz, CDCl_3): δ 1.29 (d, $J = 6.2$ Hz, 3H, 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}C NMR (75 MHz, CDCl_3): δ 16.91 (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, 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 16.²¹ 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.²² 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 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 cm^{-1} by coadding 50 scans. Samples were held in a 250 μm path
359 length cell with BaF_2 windows. IR spectra of **2a** and **2b** were
360 measured in 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 μL 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 μm , 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 CH_2Cl_2 at a
375 concentration of ~ 1 mg/L.

376 **2.11. Nuclear Magnetic Resonance Spectroscopy.** The ^1H
377 and ^{13}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 (CDCl_3) as
380 the solvent. The spectra were referenced using the lock frequency of

deuterated solvent. Chemical shifts (δ) 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

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

2.13. ODT. ODT determination experiments were carried out in
399 the sensory facility at ISVV. The ODT of **1** (commercial racemate)
400 was measured in the model wine following the ISO 13301:2018
401 guideline.²⁴ The model wine solution was freshly prepared by
402 dissolving food-grade L-(+)-tartaric acid (5 g/L) in a 12% (v/v)
403 ethanol–water solution, and the pH was adjusted to 3.2 using NaOH
404 pellets. To limit the change of the concentration of **1**, fresh standard
405 solutions of **1** were prepared, stored in an inert atmosphere at -20
406 $^\circ\text{C}$, and used for sensory analysis within 24 h. The addition of **1** to the
407 model wine solution was performed 1 h before the sensory sessions. A
408 panel of 25 participants (M 12, F 13, average age: 31, students and
409 researchers at ISVV) were presented with six sets of triangular tests
410 arranged in an ascending order in terms of the concentration of **1** (5,
411 15, 45, 135, 405, and 1215 ng/L). Each set consisted of three samples,
412 in which one was different from the other two. Standard clear wine
413 glasses were coded with a three-digit number, filled with 25 mL of the
414 sample, and covered with lids. The participants were instructed to
415 smell each sample within each set and to choose the sample exhibiting
416 different aroma. The panelist was asked to provide descriptors for the
417 aroma perceived. The collected sensory data were processed
418 according to the group best estimated threshold procedure outlined
419 in ISO 13301:2018.²⁴ ODT of *trans*-**1a** was determined in the same
420 manner in a separate session by the same panel within the same day. 421

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

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

3. RESULTS AND DISCUSSION

3.1. GC–MS/O Profile of Ag^+ SPE Extracts of Selected
439 **Wines.** A total of eight red wines (vintage 2001 to 2015, aged
440 8 to 22 years) from the Bordeaux region (Table 1) were
441 selected for their distinctive empyreumatic aging bouquet. To
442 search potentially new volatile thiols, the Ag^+ SPE protocol
443 recently developed in our laboratory¹⁷ was used to isolate thiol
444 fractions from red wines. Because of the trace quantity of thiols
445 in red wines and the effluent splitting between MS and O, extra 446

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 Ag⁺ SPE extracts to a
 451 small volume (≤50 μL). 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 Ag⁺ SPE extract on a DB-5MS column is
 458 shown in Figure 1a. The chemical and sensorial complexity of

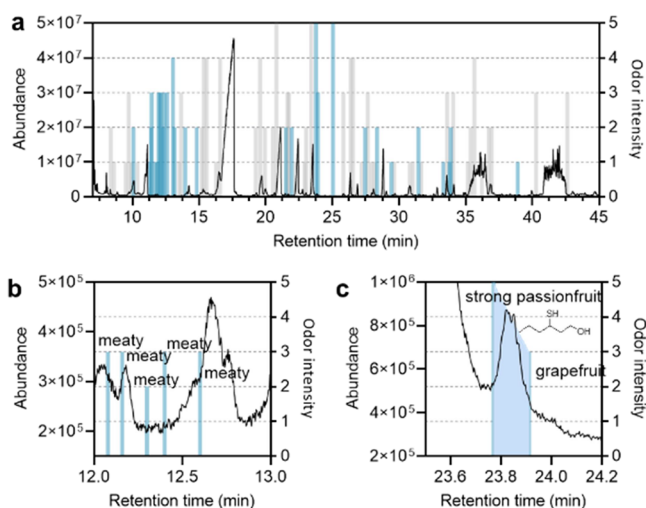


Figure 1. (a) Representative TIC overlaid with the aromagram of a Bordeaux red wine (W1) analyzed by Ag⁺ SPE and GC–MS/O fitted with a DB-5MS column (30 m × 0.25 mm i.d., 0.25 μm). (b, c) Expanded sections of (a). Vertical lines represent perceived OZs; in blue: OZs of interest, in gray: other OZs. Shaded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

459 the 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 Ag⁺ SPE sorbent used. Our observation on the affinity of
 470 Ag⁺ sorbent toward multiclass of molecules was mirrored by a
 471 recent report.²⁵ 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
 resolved peak on GC–MS/O be made, such as for 3SH
 (Figure 1c). To resolve coelutions and obtain meaningful MS/
 O information, H/C MDGC–MS/O was necessary.

3.2. H/C MDGC–MS/O Profile of Ag⁺ SPE Extracts of Selected Wines. Table S1 of the Supporting Information also presents respective OZs of interest detected after nine scheduled heart-cuts of sample W8. The first evident observation was that H/C MDGC successfully resolved persistent coelutions that were otherwise observed by conventional GC–MS/O using a single column (¹D–olfactometry). However, the odors detected after the second column by MDGC–MS/O (²D–olfactometry) were not always consistent with those perceived on the ¹D. This discrepancy in perceived odors between the ¹D separations and ²D was likely to be the results of perceptual interaction of coeluting odorants on the ¹D which were resolved by the ²D. The next obvious point was that a number of OZs reminiscent of “meaty” and “cooked food” were detected. Although “meaty” aroma has been mentioned to contribute to the aging bouquet of fine Bordeaux red wines,^{2,3,26,27} the associated molecules were limited to half a dozen known volatile thiols of which only 2M3FT exhibits a “meaty” or “roasted” odor. In another GC–MS/O study on aged red wine, FFT and 2M3FT were identified as contributing aroma compounds to the “savory” or “umami-type” aroma.²⁸ Our H/C MDGC–MS/O results clearly illustrated a deep involvement of “meaty” “cooked food” odors in the overall aroma space of premium Bordeaux red wines. Another interesting observation was that “meaty”/ “cooked food” OZs (LRI^{DB-WAX} ~ 964) occurred in all nine H/C runs despite being derived from different heart-cuts. Regardless, most resolved chromatographic peaks on the ²D were still extremely small, which is not surprising for trace thiol analytes. In many instances, meaningful mass spectra for intriguing odors were still not obtainable. With effortful manual peak picking and mass spectrum interpretation, the following previously known thiols 3-sulfanyl-2-methylpropan-1-ol, ethyl 2-sulfanylacetate, 3SH, 4MSP, 2M3FT, and FFT were found in the selected Bordeaux red wines. Certain detected thiols have been particularly associated with the positive aroma expression (3SH, 2M3FT, and FFT) of aged red wines^{2,28} and empyreumatic notes (FFT) in various wines.^{12,13,28}

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

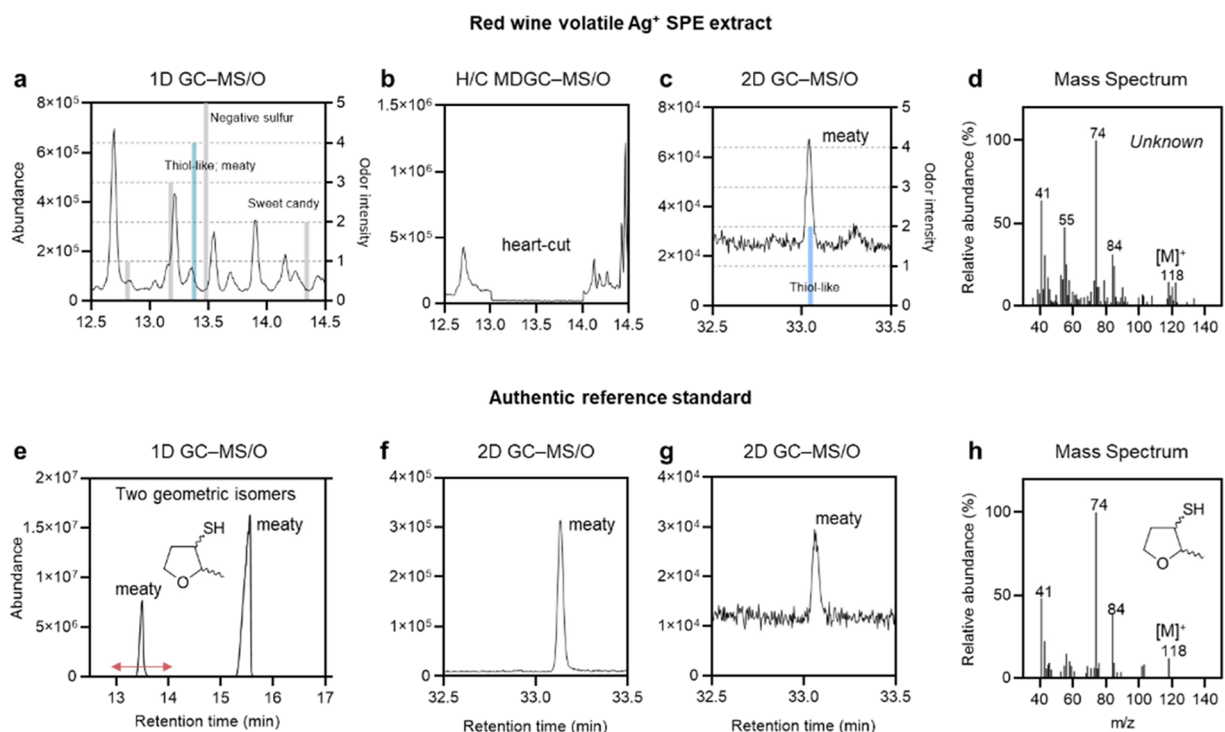


Figure 2. Compiled H/C MDGC–MS/O identification results of one isomer of 2-methyltetrahydrofuran-3-thiol **1** in a 2007 Bordeaux red wine (W3). (a) A segment (12.5–14.5 min) of GC–MS/O TIC of a wine Ag⁺ SPE extract overlaid with detected OZs showing a distinct “thiol-like” “meaty” odor with an intensity 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).

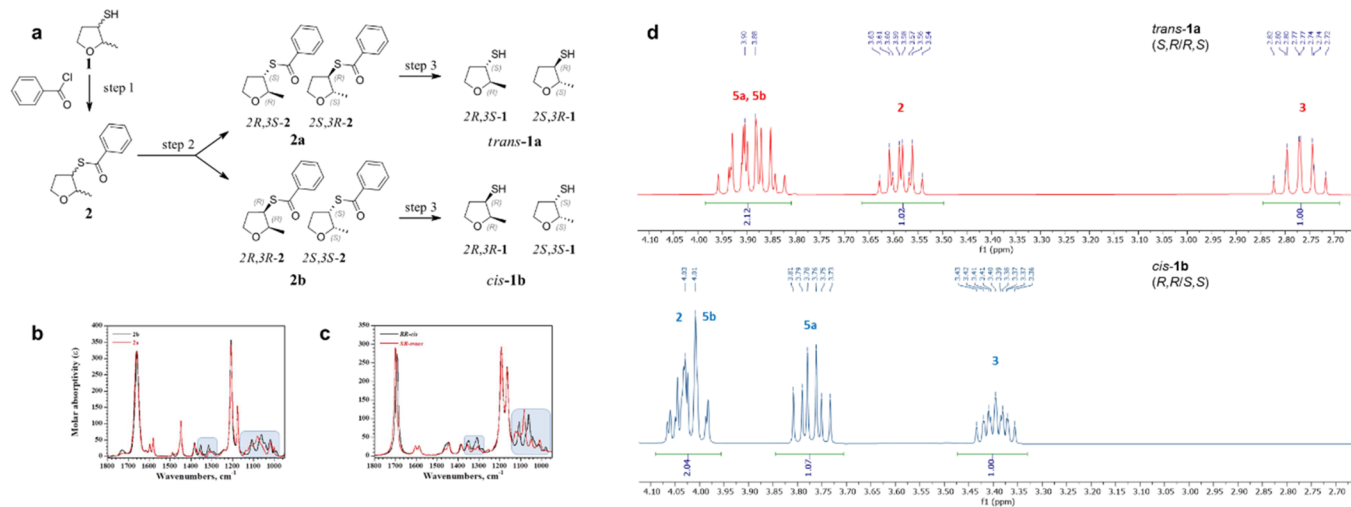


Figure 3. (a) Chemical synthesis routes to pure *trans* (*trans-1a*) and *cis* (*cis-1b*) isomers of 2-methyltetrahydrofuran-3-thiol from commercial **1**. Step 1: benzoyl chloride, K₂CO₃, acetone, rt, 3.5h. Step 2: flash chromatography (*n*-pentane/Et₂O, 1/9, v/v). Step 3: LiOH, H₂O, rt, 5h. (b) Experimental 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 ¹H NMR spectra of H2, H3, and HS 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⁺ SPE H/C MDGC–MS/O.** A
 548 pleasant “meaty”/“roasted meat” odor was noticed at
 549 ~LRI^{DB-SMS} 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^{DB-SMS} 900, 552
 Figure 2b) was performed. A total of six OZs were perceived 553
 on the ²D of which one had the same “cooked/roasted meat” 554
 odor that was noted on GC–MS/O. A fully resolved 555

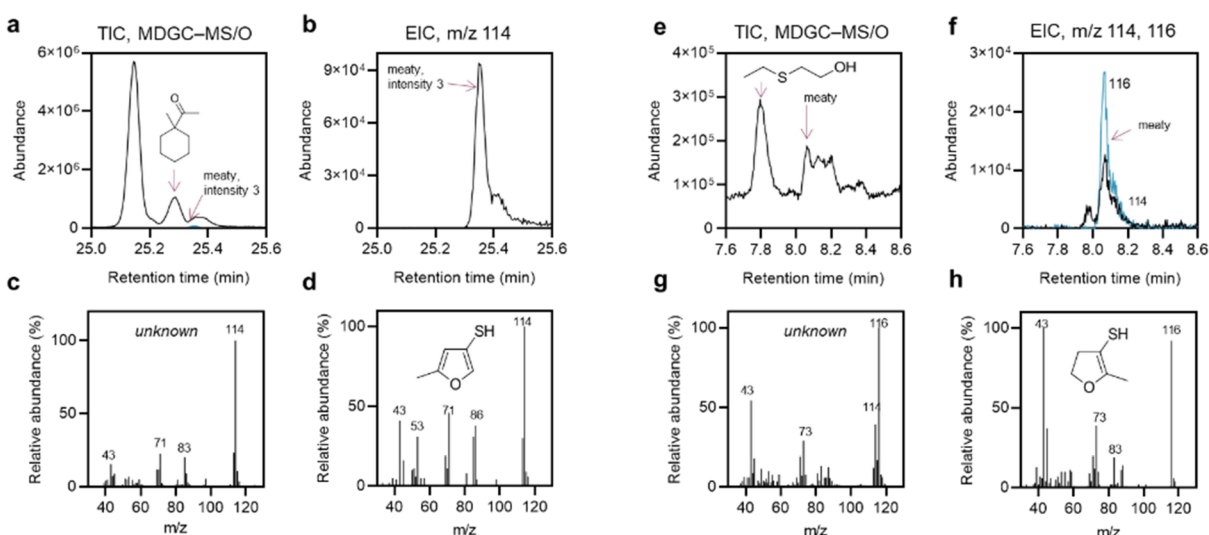


Figure 4. Chromatography and mass spectra of two unknown “meaty” smelling odors detected in the Ag^+ SPE extracts of selected Bordeaux red wines (W5 and W3) and two reference mass spectra. (a, e) TICs of the ^2D showing the respective retention regions; (b, f) EICs of the ^2D revealing the detection 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 ^2D (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 flavorings³⁰ and coffee products^{31,32} in a few cases. In a
 569 previously published review,³³ 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.^{34,35} 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 air³⁶). 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 ^1H 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,³⁷ four enantiomers of **1** possessed distinguishable

odor properties and different ODTs ($\sim\text{pg}$ levels, in the 595
 unspecified matrix). Therefore, the ratio of *cis/trans* of **1** in the 596
 commercial racemate was determined to confirm the peak 597
 identity. NMR analysis of the commercial *cis/trans* mixture of 598
1 was carried out, but the spectrum was too difficult to 599
 interpret and did not allow confidently distinguishing between 600
 the two pairs of enantiomers (data not shown). Therefore, 601
 chemical synthesis (Figure 3a) was conducted to separate *cis*- 602
1b and *trans*-**1a** from the commercial mixture. The chemical 603
 modification of the sulfhydryl group in **1** was achieved by the 604
 reaction with benzoyl chloride to form thioester **2**. The steric 605
 hindrance caused by the thioester formation facilitated the 606
 chromatographic separation of the *cis/trans* mixture by classical 607
 flash chromatography on silica gel to give two fractions: **2a** and 608
2b. The fractions **2a** and **2b** were studied by IR spectroscopy 609
 to discriminate the two pairs of *cis* (*RR/SS*) and *trans* (*SR/RS*) 610
 diastereomers. IR spectra recorded in CDCl_3 solution at a 611
 concentration of 60 mM are reported in Figure 3b in the 612
 1800–950 cm^{-1} spectral range. The IR spectra of **2a** and **2b** 613
 are similar, except around 1300 cm^{-1} and in the 1150–1000 614
 cm^{-1} region. The band around 1300 cm^{-1} is related to the 615
 bending δOCH and wagging ωCH_2 of the furan group, 616
 whereas the bands in the 1150–1000 cm^{-1} region are related 617
 to the asymmetric $\nu_a\text{COC}$ and symmetric $\nu_s\text{COC}$ stretching 618
 modes of the furan group coupled with rocking CH_2 and CH_3 . 619
 The bands associated with the thio-benzoyl group are not 620
 sensitive to the stereochemistry of the molecule ($\nu\text{C}=\text{O}$ at 621
 1660 cm^{-1} , $\nu_{8a}\text{C}=\text{C}$ and $\nu_{8b}\text{C}=\text{C}$ at 1598 and 1582 cm^{-1} , 622
 $\nu_{19a}\text{C}=\text{C}$ and $\nu_{19b}\text{C}=\text{C}$ at 1489 and 1449 cm^{-1} , $\delta_{\text{ip}}\text{CH}$ of 623
 phenyl at 1208 and 1177 cm^{-1}). 624

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

633 The ^1H 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 ^1H 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 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 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 ^1D
668 at $\sim \text{LRI}^{\text{DB-SMS}} 937$ (rt = 15.20 min) of five bottles of WS. 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 ^2D , 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.¹⁹ The same authors also
694 suggested¹⁹ 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
reaction was proposed to be 2-methyl-4-furanthiol (2M4FT,
no LRI information available),³⁸ another isomer to 2M3FT.
Without a pure reference standard, only tentative identification
can be reached. None of the suspected isomers to 2M3FT
were commercially available, and several attempts to synthesize
5M3FT were made, but none was successful (data not shown).

Another strong-smelling ($I = 5$) “meaty” odor was detected
in the Ag^+ SPE extract (20 μL) prepared from W3 at
 $\sim \text{LRI}^{\text{DB-SMS}} 941$. To enhance detectability, repeated injections
($n = 5$) were performed, and five heart-cuts were cryogenically
focused on the head of the ^2D column and released at once for
MDGC–MS/O analyses. TIC shows persistent coelutions
(Figure 4e). A small but partially resolved peak was noticed for
the “meaty” aroma ($I = 4$) on the ^2D . Ions m/z 114 and 116
were observed at the corresponding retention time (Figure 4f).
A clean mass spectrum was obtained for this partially resolved
peak (Figure 4g). Again, no probable hits could be retrieved
from the NIST 2014 library. After comparison to the same
external database (Firmenich), the peak identity was indicated
as 4,5-dihydro-2-methyl-3-furanthiol (DH2M3FT). The
archived reference mass spectrum of DH2M3FT is provided in
Figure 4h. This thiofuran was also reported as the volatile
component from Maillard reaction that had “roasted meat”
aromas.^{38–40} DH2M3FT was previously patented as a flavoring
substance⁴¹ and its formation pathways were proposed.⁴²
DH2M3FT had LRI values reported as 927⁴⁰ on a DB-1
column and 939³⁹ on a HP-5MS column, which was close to
our data (941 on a DB-SMS column). Substantial efforts for its
synthesis were devoted but eventually proved fruitless in
producing this targeted thiol even at a trace quantity (data not
shown). Alternatively, we investigated the possibility of
generating these two thiofurans (DH2M3FT and 5M3FT)
through the Maillard reaction, which was known to produce
“meaty” aromas. Following a literature protocol,¹⁹ a Maillard
reaction was performed. The resulting Maillard reaction
mixture exhibited extremely intense “meaty” aromas. Although
the mass spectra very similar to those of suspected thiofurans
(Figure 4d,h) were noticed in the Maillard reaction mixture by
GC–MS/O, due to the restraint on time and resources, the
isolation of targeted thiol fractions from the Maillard reaction
mixture and subsequent identification were not pursued.
Therefore, the structural identify of these two “meaty” smelling
compounds remains to be elucidated upon obtaining pure
reference standards. The access to the unavailable standards
may be achieved by novel chemical synthesis by fractionating
Maillard reaction mixture using Ag^+ SPE and preparative scale
MDGC with a cryogenic fraction trap, or by other alternative
means.⁴³ Although Ag^+ SPE with preparative MDGC for
isolating trace thiofurans from Maillard reaction extracts is
without precedent, the concept of the technique is well-known
for small molecule discovery.⁴⁴ One analytical system adapting
single- dimension preparative GC with the fraction collector
has already allowed the separation, collection, and identi-
fication of volatile compounds in wine.⁴⁵

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

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^{14,15} and coffee,^{15,32} in which various
766 sample cleanup protocols were applied. Here, Ag⁺ 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⁺ SPE extracts (first CH₂Cl₂ washing and the
772 final elute) prepared from the same wines were collected.
773 According to previous data,¹⁷ 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⁺ 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⁺ 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,¹⁵ thiol-rich Sauvignon blanc wine,⁴⁶ and other
792 wines,¹⁴ a considerably higher number of thiols reported in this
793 study clearly suggests the superiority of applying Ag⁺ 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.⁴⁷ 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.¹⁶

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).³⁷ As detailed sensory

protocol and matrix information for the previous measure- 822
ments were not provided,³⁷ 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,²⁶ 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.⁴⁸ 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.³⁷ Influence 842
of structural modification and chirality on the odor quality of 843
thiols was well documented.³⁶ 844

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.²⁶ 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 10¹⁴ parts of water.⁴⁹ The high reactivity of 2M3FT has 860
been long known,²⁶ and it can be easily oxidized to its disulfide 861
form.¹⁷ 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.⁵⁰ 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)^a 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)

^aData 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⁺ 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.³¹ Regardless of the detected isomer(s),³¹ *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.^{2,26} 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⁺ 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**; ¹H 925 and ¹³C 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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998 ■ **REFERENCES**

- 999 (1) Bouchilloux, P.; Darriet, P.; Henry, R.; Lavigne-Cruège, V.;
1000 Dubourdieu, D. Identification of Volatile and Powerful Odorous
1001 Thiols in Bordeaux Red Wine Varieties. *J. Agric. Food Chem.* **1998**, *46*
1002 (8), 3095–3099.
- 1003 (2) Picard, M.; Thibon, C.; Redon, P.; Darriet, P.; de Revel, G.;
1004 Marchand, S. Involvement of Dimethyl Sulfide and Several Polyfunc-
1005 tional Thiols in the Aromatic Expression of the Aging Bouquet of Red
1006 Bordeaux Wines. *J. Agric. Food Chem.* **2015**, *63* (40), 8879–8889.
- 1007 (3) Picard, M.; Tempere, S.; de Revel, G.; Marchand, S. A Sensory
1008 Study of the Ageing Bouquet of Red Bordeaux Wines: A Three-Step
1009 Approach for Exploring a Complex Olfactory Concept. *Food Qual.*
1010 *Prefer.* **2015**, *42*, 110–122.
- 1011 (4) Culleré, Laura; Escudero, A.; Cacho, J.; Ferreira, V. Gas
1012 Chromatography–Olfactometry and Chemical Quantitative Study of
1013 the Aroma of Six Premium Quality Spanish Aged Red Wines. *J. Agric.*
1014 *Food Chem.* **2004**, *52* (6), 1653–1660.
- 1015 (5) Chen, L.; Darriet, P. Strategies for the Identification and Sensory
1016 Evaluation of Volatile Constituents in Wine. *Compr. Rev. Food Sci.*
1017 *Food Safety* **2021**, *20* (5), 4549–4583.
- 1018 (6) Darriet, P.; Pons, A. Wine. In *Springer Handbook of Odor*;
1019 Buettner, A., Ed.; Springer Handbooks; Springer International
1020 Publishing: Cham, 2017; pp. 25–26.
- 1021 (7) Dunkel, A.; Steinhaus, M.; Kotthoff, M.; Nowak, B.; Krautwurst,
1022 D.; Schieberle, P.; Hofmann, T. Nature’s Chemical Signatures in
1023 Human Olfaction: A Foodborne Perspective for Future Biotechnol-
1024 ogy. *Angew. Chem., Int. Ed.* **2014**, *53* (28), 7124–7143.
- 1025 (8) Dubourdieu, D.; Tominaga, T. Polyfunctional Thiol Com-
1026 pounds. In *Wine Chemistry and Biochemistry*; Moreno-Arribas, M. V.;
1027 Polo, M. C., Eds.; Springer New York: New York, NY, 2009; pp.
1028 275–293.
- 1029 (9) Darriet, P.; Tominaga, T.; Lavigne, V.; Boidron, J.-N.;
1030 Dubourdieu, D. Identification of a Powerful Aromatic Component
1031 of Vitis Vinifera L. Var. Sauvignon Wines: 4-Mercapto-4-Methyl-
1032 pentan-2-One. *Flavour Fragr. J.* **1995**, *10* (6), 385–392.
- 1033 (10) Chen, L.; Capone, D. L.; Jeffery, D. W. Analysis of Potent
1034 Odour-Active Volatile Thiols in Foods and Beverages with a Focus on
1035 Wine. *Molecules* **2019**, *24* (13), 2472.
- 1036 (11) Tominaga, T.; Guimbertau, G.; Dubourdieu, D. Contribution
1037 of Benzenemethanethiol to Smoky Aroma of Certain Vitis Vinifera L.
1038 Wines. *J. Agric. Food Chem.* **2003**, *51* (5), 1373–1376.
- 1039 (12) Tominaga, T.; Guimbertau, G.; Dubourdieu, D. Role of Certain
1040 Volatile Thiols in the Bouquet of Aged Champagne Wines. *J. Agric.*
1041 *Food Chem.* **2003**, *51* (4), 1016–1020.
- 1042 (13) Espinase Nandorfy, D.; Siebert, T.; Bilogrevic, E.; Likos, D.;
1043 Watson, F.; Barter, S.; Pisaniello, L.; Kulcsar, A.; Shellie, R. A.; Keast,
1044 R.; Francis, L.; Bekker, M. The Role of Potent Thiols in
1045 “Empyreumatic” Flint/Struck-Match/Mineral Odours in Chardonnay
1046 Wine. *Austr. J. Grape Wine Res.* **2023**, *2023*, No. e8847476.
- 1047 (14) Schoenauer, S.; Schieberle, P. Screening for Novel Mercaptans
1048 in 26 Fruits and 20 Wines Using a Thiol-Selective Isolation Procedure
1049 in Combination with Three Detection Methods. *J. Agric. Food Chem.*
1050 **2019**, *67* (16), 4553–4559.
- (15) Chin, S.-T.; Eyres, G. T.; Marriott, P. J. Identification of Potent
1051 Odourants in Wine and Brewed Coffee Using Gas Chromatography-
1052 Olfactometry and Comprehensive Two-Dimensional Gas Chroma-
1053 tography. *J. Chromatogr. A* **2011**, *1218* (42), 7487–7498. 1054
- (16) Suhas, E.; Shinkaruk, S.; Pons, A. *Optimizing the Identification of*
1055 *Thiols in Red Wines Using New Oak-Wood Accelerated Reductive*
1056 *Treatment*. Rochester, NY, August 14, 2023. DOI: 10.2139/
1057 *ssrn.4538398*. 1058
- (17) Chen, L.; Darriet, P. Qualitative Screening of Volatile Thiols in
1059 Wine by Selective Silver Ion Solid-Phase Extraction with Heart-
1060 Cutting Multidimensional Gas Chromatography Mass Spectrometry/
1061 Olfactometry. *J. Agric. Food Chem.* **2022**, *70* (15), 4701–4711. 1062
- (18) Mitra, S.; Brukh, R. Sample Preparation: An Analytical
1063 Perspective. In *Sample Preparation Techniques in Analytical Chemistry*;
1064 John Wiley & Sons, Ltd, 2003; pp. 1–36. 1065
- (19) Farmer, L. J.; Mottram, D. S.; Whitfield, F. B. Volatile
1066 Compounds Produced in Maillard Reactions Involving Cysteine,
1067 Ribose and Phospholipid. *J. Sci. Food Agric.* **1989**, *49* (3), 347–368. 1068
- (20) Sasamoto, K.; Ochiai, N. Selectable One-Dimensional or Two-
1069 Dimensional Gas Chromatography–Mass Spectrometry with Simul-
1070 taneous Olfactometry or Element-Specific Detection. *J. Chromatogr. A*
1071 **2010**, *1217* (17), 2903–2910. 1072
- (21) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.;
1073 Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Petersson,
1074 G. A.; Nakatsuji, H. *Gaussian 16*, 2016. Gaussian Inc.: Wallingford,
1075 CT, 2016. 1076
- (22) Dennington, R.; Keith, T. A.; Millam, J. M. *GaussView 6.0*. 16.
1077 Semichem Inc.: Shawnee Mission, KS, USA, 2016. 1078
- (23) Capone, D. L.; Ristic, R.; Pardon, K. H.; Jeffery, D. W. Simple
1079 Quantitative Determination of Potent Thiols at Ultratrace Levels in
1080 Wine by Derivatization and High-Performance Liquid Chromatog-
1081 raphy–Tandem Mass Spectrometry (HPLC-MS/MS) Analysis. *Anal.*
1082 *Chem.* **2015**, *87* (2), 1226–1231. 1083
- (24) ISO. ISO 13301:2018 Sensory Analysis — Methodology —
1084 General Guidance for Measuring Odour, Flavour and Taste Detection
1085 Thresholds by a Three-Alternative Forced-Choice (3-AFC) Proce-
1086 dure. 2018. 1087
- (25) Pua, A.; Yeam, C. W.; Huang, Y.; Goh, R. M. V.; Ee, K.-H.;
1088 Lassabliere, B.; Liu, S. Q.; Yu, B. Sequential Combination of Solid-
1089 Phase Sorbents to Enhance the Selectivity of Organosulfur
1090 Compounds for Flavour Analysis. *Talanta* **2022**, *241*, No. 123234. 1091
- (26) Tominaga, T.; Dubourdieu, D. A Novel Method for
1092 Quantification of 2-Methyl-3-Furanthiol and 2-Furanmethanethiol in
1093 Wines Made from Vitis Vinifera Grape Varieties. *J. Agric. Food Chem.*
1094 **2006**, *54* (1), 29–33. 1095
- (27) Tominaga, T.; Blanchard, L.; Darriet, P.; Dubourdieu, D. A
1096 Powerful Aromatic Volatile Thiol, 2-Furanmethanethiol, Exhibiting
1097 Roast Coffee Aroma in Wines Made from Several Vitis Vinifera Grape
1098 Varieties. *J. Agric. Food Chem.* **2000**, *48* (5), 1799–1802. 1099
- (28) Beatty, A. M. *Characterization of “Savory” Aroma Compounds in*
1100 *Aged Red Wines via Gas Chromatography-Olfactometry and Descriptive*
1101 *Analysis*. M.S., Purdue University: United States, Indiana, 2013. 1102
[https://www.proquest.com/docview/1477862887/abstract/](https://www.proquest.com/docview/1477862887/abstract/3FCB03AB150F4A8EPQ/1)
1103 [3FCB03AB150F4A8EPQ/1](https://www.proquest.com/docview/1477862887/abstract/3FCB03AB150F4A8EPQ/1) (accessed 2023–08–19). 1104
- (29) Vichi, S.; Cortés-Francisco, N.; Caixach, J. Analysis of Volatile
1105 Thiols in Alcoholic Beverages by Simultaneous Derivatization/
1106 Extraction and Liquid Chromatography–High Resolution Mass
1107 Spectrometry. *Food Chem.* **2015**, *175*, 401–408. 1108
- (30) Evers, W. J. Certain Furan-3-Thiols, Certain Dihydro
1109 Derivatives Thereof and 2,5-Dimethyltetrahydrofuran-3-Thiol.
1110 US4055578A, October 25, 1977. [https://patents.google.com/](https://patents.google.com/patent/US4055578A/en)
1111 [patent/US4055578A/en](https://patents.google.com/patent/US4055578A/en) (accessed 2023–03–30). 1112
- (31) Quintanilla-Casas, B.; Dulsat-Serra, N.; Cortés-Francisco, N.;
1113 Caixach, J.; Vichi, S. Thiols in Brewed Coffee: Assessment by Fast
1114 Derivatization and Liquid Chromatography–High Resolution Mass
1115 Spectrometry. *LWT - Food Sci. Technol.* **2015**, *64* (2), 1085–1090. 1116
- (32) Pua, A.; Huang, Y.; Vivian Goh, R. M.; Ee, K.-H.; Li, L.;
1117 Cornuz, M.; Lassabliere, B.; Jublot, L.; Liu, S. Q.; Yu, B. 1118
1119 Multidimensional Gas Chromatography of Organosulfur Compounds 1119

- 1120 in Coffee and Structure–Odor Analysis of 2-Methyltetrahydrothio-
1121 phen-3-One. *J. Agric. Food Chem.* **2023**, *71* (10), 4337–4345.
- 1122 (33) Wamhoff, H.; Gribble, G. W. Chapter 3 - Wine and
1123 Heterocycles. In *Adv. Heterocycl. Chem.*; Katritzky, A. R., Ed.;
1124 Advances in Heterocyclic Chemistry; Academic Press, 2012; Vol.
1125 *106*, pp. 185–225.
- 1126 (34) Grosch, W. Evaluation of the Key Odorants of Foods by
1127 Dilution Experiments, Aroma Models and Omission. *Chem. Senses*
1128 **2001**, *26* (5), 533–545.
- 1129 (35) Mateo-Vivaracho, L.; Cacho, J.; Ferreira, V. Improved Solid-
1130 Phase Extraction Procedure for the Isolation and in-Sorbent
1131 Pentafluorobenzyl Alkylation of Polyfunctional Mercaptans: Opti-
1132 mized Procedure and Analytical Applications. *J. Chromatogr. A* **2008**,
1133 *1185* (1), 9–18.
- 1134 (36) Schoenauer, S.; Schieberle, P. Structure–Odor Correlations in
1135 Homologous Series of Mercapto Furans and Mercapto Thiophenes
1136 Synthesized by Changing the Structural Motifs of the Key Coffee
1137 Odorant Furan-2-Ylmethanethiol. *J. Agric. Food Chem.* **2018**, *66* (16),
1138 4189–4199.
- 1139 (37) Goeke, A. Diastereo- and Enantioselective Syntheses of 2-
1140 Methyl-Tetrahydrofuran-3-Thiol. *Phosphorus Sulfur Silicon Relat.*
1141 *Elem.* **1999**, *153* (1), 303–304.
- 1142 (38) Van den Ouweland, G. A. M.; Peer, H. G. Components
1143 Contributing to Beef Flavor. Volatile Compounds Produced by the
1144 Reaction of 4-Hydroxy-5-Methyl-3(2H)-Furanone and Its Thio
1145 Analog with Hydrogen Sulfide. *J. Agric. Food Chem.* **1975**, *23* (3),
1146 501–505.
- 1147 (39) Cerny, C. Origin of Carbons in Sulfur-Containing Aroma
1148 Compounds from the Maillard Reaction of Xylose, Cysteine and
1149 Thiamine. *LWT - Food Sci. Technol.* **2007**, *40* (8), 1309–1315.
- 1150 (40) Guentert, M.; Bruening, J.; Emberger, R.; Koepsel, M.; Kuhn,
1151 W.; Thielmann, T.; Werkhoff, P. Identification and Formation of
1152 Some Selected Sulfur-Containing Flavor Compounds in Various Meat
1153 Model Systems. *J. Agric. Food Chem.* **1990**, *38* (11), 2027–2041.
- 1154 (41) Gerardus, P. H.; Maria, V. D. O. G. Flavor Substances and
1155 Processes for Their Production. DE1932800A1, January 8, 1970.
1156 <https://patents.google.com/patent/DE1932800A1/en> (accessed
1157 2023–07–24).
- 1158 (42) Clydesdale, F. M.; Ho, C.; Lee, C. Y.; Mondy, N. I.; Shewfelt,
1159 R. L.; Lee, K. The Effects of Postharvest Treatment and Chemical
1160 Interactions on the Bioavailability of Ascorbic Acid, Thiamin, Vitamin
1161 a, Carotenoids, and Minerals. *Crit. Rev. Food Sci. Nutr.* **1991**, *30* (6),
1162 599–638.
- 1163 (43) Bucar, F.; Wube, A.; Schmid, M. Natural Product Isolation –
1164 How to Get from Biological Material to Pure Compounds. *Nat. Prod.*
1165 *Rep.* **2013**, *30* (4), 525–545.
- 1166 (44) Eyres, G. T.; Urban, S.; Morrison, P. D.; Dufour, J.-P.; Marriott,
1167 P. J. Method for Small-Molecule Discovery Based on Microscale-
1168 Preparative Multidimensional Gas Chromatography Isolation with
1169 Nuclear Magnetic Resonance Spectroscopy. *Anal. Chem.* **2008**, *80*
1170 (16), 6293–6299.
- 1171 (45) Pons, A.; Lavigne, V.; Darriet, P.; Dubourdiou, D. Identification
1172 and Analysis of Piperitone in Red Wines. *Food Chem.* **2016**, *206*,
1173 191–196.
- 1174 (46) Beckner Whitener, M. E.; Stanstrup, J.; Panzeri, V.; Carlin, S.;
1175 Divol, B.; Du Toit, M.; Vrhovsek, U. Untangling the Wine
1176 Metabolome by Combining Untargeted SPME–GCxGC–TOF–MS
1177 and Sensory Analysis to Profile Sauvignon Blanc Co-Fermented with
1178 Seven Different Yeasts. *Metabolomics* **2016**, *12* (3), 53.
- 1179 (47) Tomasino, E.; Cerrato, D. C.; Aragon, M.; Fryer, J.; Garcia, L.;
1180 Ashmore, P. L.; Collins, T. S. A Combination of Thiophenols and
1181 Volatile Phenols Cause the Ashy Flavor of Smoke Taint in Wine. *Food*
1182 *Chem. Adv.* **2023**, *2*, No. 100256.
- 1183 (48) Culleré, L.; Escudero, A.; Pérez-Trujillo, J. P.; Cacho, J.;
1184 Ferreira, V. 2-Methyl-3-(Methyldithio)Furan: A New Odorant
1185 Identified in Different Monovarietal Red Wines from the Canary
1186 Islands and Aromatic Profile of These Wines. *J. Food Compos. Anal.*
1187 **2008**, *21* (8), 708–715.
- (49) Buttery, R. G.; Haddon, W. F.; Seifert, R. M.; Turnbaugh, J. G. 1188
Thiamin Odor and Bis(2-Methyl-3-Furyl) Disulfide. *J. Agric. Food* 1189
Chem. **1984**, *32* (3), 674–676. 1190
- (50) Bailly, S.; Jerkovic, V.; Marchand-Brynaert, J.; Collin, S. Aroma 1191
Extraction Dilution Analysis of Sauternes Wines. Key Role of 1192
Polyfunctional Thiols. *J. Agric. Food Chem.* **2006**, *54* (19), 7227– 1193
7234. 1194