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First synthesis of 3-S-glutathionylhexanal-d_8 and its bisulfite adduct

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ABSTRACT

3-Sulfanylhexan-1-ol (3SH) is an impact odorant of white wines, imparting tropical fruit aromas. A reliable synthetic pathway to 3-S-glutathionylhexanal (glut-3SH-al), a precursor to 3SH that has not been intensively studied, was developed starting from 1-butanol. Application of this synthesis to 1-butanol-d_{10}, conserved eight deuteriums, producing glut-3SH-al-d_8, which can be used as an internal standard for future work on the occurrence and evolution of glut-3SH-al in wine systems. Additionally, both glut-3SH-SO_3 and glut-3SH-SO_3-d_8 were synthesised from the corresponding aldehyde, enabling further study of the role of these bisulfite adducts in 3SH biogenesis.

Key impact odorants of white wines, particularly those made from *Vitis vinifera* L. cv. Sauvignon blanc grapes, are three volatile thiol compounds 4-methylsulfanylpentan-2-one (**1**, 4MSP), 3-sulfanylhexan-1-ol (**2**, 3SH), and 3-sulfanylhexyl acetate (**3**, 3SHA) (see Fig. 1) [1–4].

These compounds contribute significantly to the aromatic profile of white wines and manipulation of the concentration of these compounds results in measurable changes to wine aroma [5].

Of particular interest are 3SH **2** and 3SHA **3** which show a 95% correlation between their concentration and the associated sensory descriptors of grapefruit, and sweet, sweaty passionfruit, which are desirable characters in wines [2]. In wine systems, 3SH and 3SHA exist as a scalemic mixture of the *R* and *S* enantiomers, with each enantiomer having slightly different perception thresholds and aromas [6]. Volatile thiol aroma compounds such as 3SH **2** are produced during fermentation from precursors present in the grape must, including glutathione and/or cysteine conjugates of C-6 compounds such as 3-S-glutathionylhexanol (**4**, glut-3SH) and 3-S-cysteinylhexanol (**5**, cys-3SH) [7]. As with the volatile thiols, these precursors exist in a mixture of diastereomers with variable stereochemistry at the C-3 position of the C-6 moiety [6]. Formation of these precursors, and their conversion to 3SH **2** [6] occurs through a combination of enzymatic and chemical reactions, leading to a complex pathway (see Scheme 1) [8]. The current understanding of this pathway explains less than 50% of the final 3SH **2** concentrations in wine, suggesting that there is potential for further elucidation of 3SH **2** in wine than what is currently established [9].

One group of precursors to 3SH **2** which have been under-examined are the glutathione conjugate of *E*-2-hexenal **6**, the aldehyde derivative of glut-3SH **4**, 3-S-glutathionylhexanal (**7**, glut-3SH-al), and its bisulfite adduct (**8**, glut-3SH-SO_3), highlighted in Scheme 1 [8–11].

Glut-3SH-al **7** has been previously identified in both juice and finished wine [8,10,11]. Clark and Deed [8] found that glut-3SH-al **7** was produced in appreciable levels by the chemical addition of glutathione to *E*-2-hexenal **6** in grape must. Although the synthesis of glut-3SH-al **7** and glut-3SH-SO_3 **8** have been previously reported, these synthetic routes began from commercially-available *E*-2-hexenal **6**, which is not readily available in deuterated form [10,11]. The lack of commercially-available deuterated *E*-2-hexenal **6** precludes the synthesis of highly-deuterated analogues of glut-3SH-al **7**, which are important for further elucidation of the role of glut-3SH-al **7** in the formation of 3SH **2**. While glut-3SH-al-d_8 has been reportedly identified in grape must spiked with *E*-2-hexenal-d_8 **6-d_8**, the synthesis and full characterisation of this compound has not yet been reported [12]. This work reports the development of a synthetic pathway to glut-3SH-al **7** and its highly-deuterated analogue, glut-3SH-al-d_8 **7-d_8**, along with their bisulfite adducts. We are the first to report the synthesis of glut-3SH-al-d_8 **7-d_8** and glut-3SH-SO_3-d_8 **8-d_8.**

Synthesis of glut-3SH-al **7** began with the generation of butanal **9** and, subsequently, *E*-2-ethyl hexenoate **11a** from commercially-available 1-butanol **10** (see Scheme 2) as reported by Jelley and co-workers [13]. Butanal **9** was obtained through a Swern oxidation

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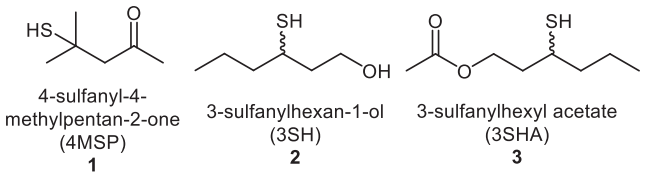


Fig. 1. The structures of key thiol aroma compounds.

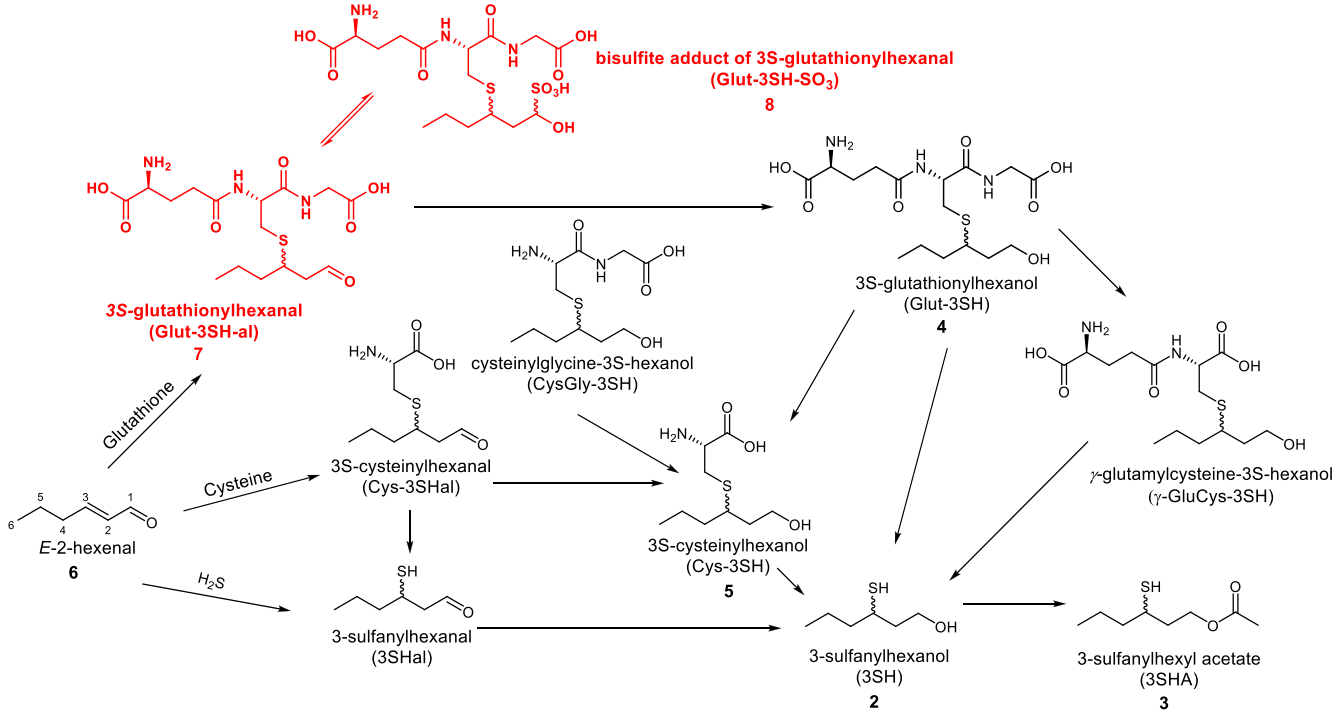
with oxalyl chloride at −78 °C and was subsequently reacted without further purification due to its volatility. A selective Wittig olefination between butanal **9** and (carbethoxymethylene) triphenylphosphorane was carried out and purification by flash chromatography yielded both *E*-ethyl 2-hexenoate **11a** and *Z*-ethyl 2-hexenoate **11b**, in separate fractions, in an isomeric ratio of 95:5. Reduction of *E*-ethyl 2-hexenoate **11a** to *E*-2-hexen-1-ol **12** was achieved using diisobutylaluminium hydride (DIBAL) at −78 °C and reaction side products were removed by washing with a saturated solution of Rochelle's salt. Purification was carried out using flash chromatography and the resulting alcohol was obtained in 79% yield, before being oxidised to the corresponding aldehyde using pyridinium chlorochromate. *E*-2-hexenal **6** was obtained as a pale-yellow solution, and the crude product was used directly in the next reaction due to the volatility of the product. Addition of the glutathione moiety was carried out as described by Thibon and co-workers [10] and the resulting pale orange powder was collected. Portions of the crude product were purified on prepacked Supelclean™ ENVI™-18 SPE tubes with an ethanol/water solvent system. Fractions containing **7** were freeze-dried and the resulting white powder was characterised as **7** (23%). Both NMR and HRMS data of glut-3SH-al **7** was in agreement with previous literature [10].

The deuterated analogues of these compounds were obtained through the same reaction conditions, beginning from 1-butanol-d_{10} **9-*d*$_{10}$**, with comparable yields for every step (see Scheme 3). HRMS data for glut-3SH-al-d_8 **7-*d*$_8$** confirmed the inclusion of eight deuterium atoms, while ^{1}H and ^{13}C NMR spectra were in agreement with the spectra for glut-3SH-al **7**, apart from missing peaks corresponding to replacement of ^{1}H with ^{2}H on C-6, C-5, C-4, and C-3.

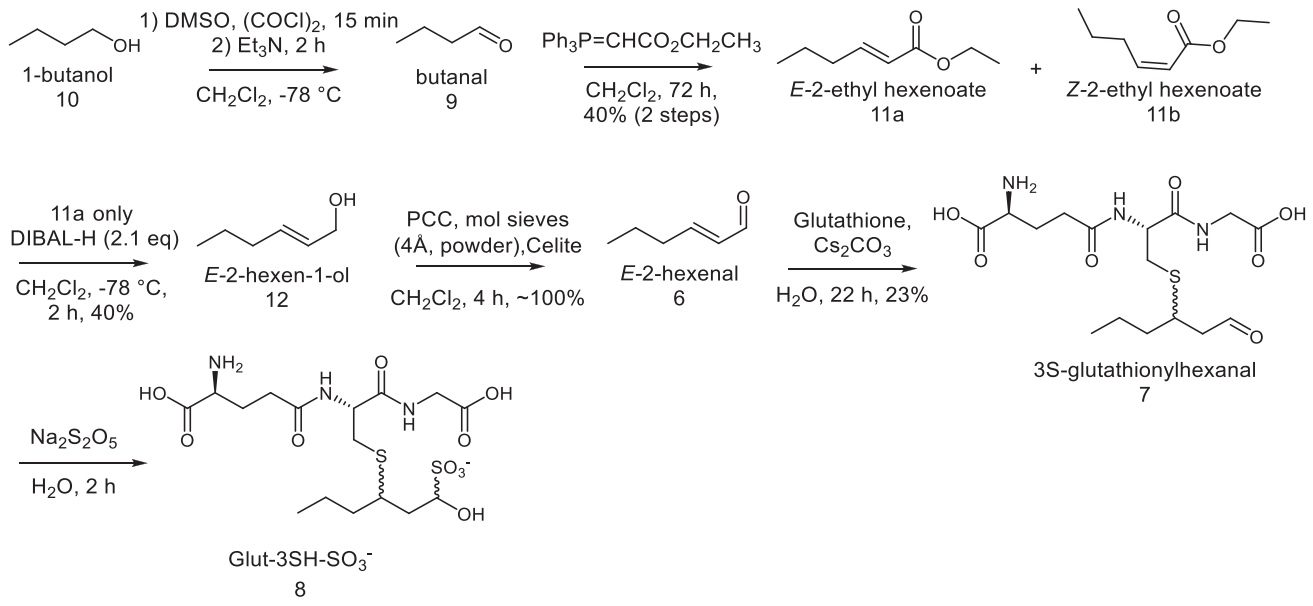
Additionally, the fragmentation pattern observed in the HRMS spectrum for glut-3SH-al-d_8 **7-*d*$_8$** was in agreement with that reported by Capone and Jeffery [12], confirming their tentative identification of glut-3SH-al-d_8 **7-*d*$_8$** in juice from grapes spiked with *E*-2-hexenal-d_8 **6-*d*$_8$**.

The synthesis of the bisulfite adduct of glut-3SH-al **7**, glut-3SH-SO_3 **8** has been previously reported in the literature, along with its identification in samples of grape juice (and wine) [8,10,11]. However, the isotopically labelled version, glut-3SH-SO_3-d_8 **8-*d*$_8$** has never been reported, and will be of great use in analytical studies investigating the interconversion of glut-3SH-SO_3 **8** and glut-3SH-al **7** in grape juice and wine. Both glut-3SH-SO_3 **8** and glut-3SH-SO_3-d_8 **8-*d*$_8$** were synthesised for NMR analysis by addition of sodium metabisulfite to a sample of purified glut-3SH-al **7** in D_2O 30 min prior to analysis – the NMR of the synthesised product glut-3SH-SO_3 **8** was in accordance with previously reported data [10], while the NMR of the deuterated analogue glut-3SH-SO_3-d_8 **8-*d*$_8$** was in agreement with its non-deuterated counterpart **8**. For further analysis, the bisulfite adducts were synthesised by mixing an aqueous solution of $Na_2S_2O_5$ (1 equivalent) with an aqueous solution of the aldehyde. This enabled MS analysis of the product, which confirmed the synthesis of **8**. HRMS of **8** was in agreement with the data reported by Thibon and co-workers [10], as was **8-*d*$_8$**, though with a mass increase of 8.

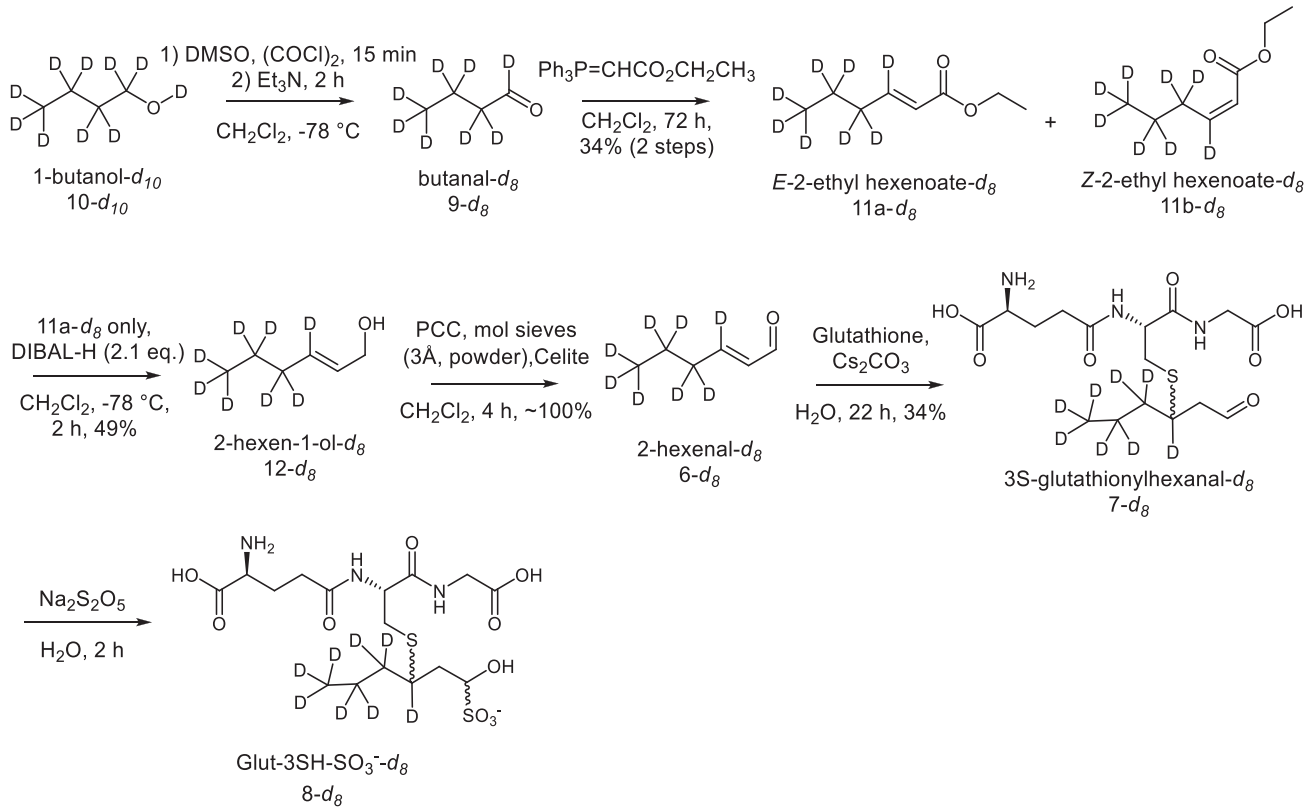
In summary, we report the synthesis of glut-3SH-al **7** using a novel pathway, beginning from 1-butanol **9**, as well as the first total synthesis of glut-3SH-al-d_8 **7-*d*$_8$**, a critical compound for the analysis of 3SH **2** formation in wines. The new synthetic approach to generate glut-3SH-al **7** from 1-butanol **9** had not been previously reported and permitted the synthesis of the heavily deuterated glut-3SH-al-d_8 **7-*d*$_8$** from 1-butanol-d_{10} **9-*d*$_{10}$**. The incorporation of eight deuterium atoms in glut-3SH-al-d_8 **7-*d*$_8$**



Scheme 1. Current understanding of 3SH biogenesis pathways in wine. Compounds synthesised in this work are highlighted in red.



Scheme 2. Synthesis of compound **7** and **8**, starting with butanol **10**.



Scheme 3. Synthesis of compounds **7-*d*$_8$** and **8-*d*$_8$** starting from commercially available butanol-d_{10} **10-*d*$_{10}$**.

reported here has the potential to be extremely useful for mechanistic studies on glut-3SH-al **7** breakdown in wine, as well as the analysis of glut-3SH-al **7** concentrations, since the loss of a single deuterium atom will not result in the compound being indistinguishable from the natural glut-3SH-al **7**. Additionally, the deuterium atoms are incorporated in the hexenal moiety, the section of interest in the breakdown of glut-3SH-al **7** during fermentation. Use of glut-3SH-al-d_8 **7-*d*$_8$** for metabolomic studies would result in all breakdown products through to 3SH containing the eight deuterium atoms and therefore able to be distinguished from the natural products. Synthesis and isolation of glut-3SH-al-d_8 **7-*d*$_8$** enables the use of this compound as an internal standard for further studies of the formation of 3SH **2** *via* metabolism of the glut-3SH-al-d_8 **7-*d*$_8$**. Additionally, this enabled the synthesis of

glut-3SH-SO_3-d_8 **8-d_8** for the first time. This compound has not been reported previously, either synthetically or in spiked grape must, and will enable further elucidation of the place of bisulfite additions to aldehydes in the grape must prior to, during, and after, fermentation.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tetlet.2020.152100.

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