COMMUNICATION

Direct Arene Trifluoromethylation Enabled by Promiscuous Activity of Fungal Laccase

Yi Ling Goh,[†a] Shi Yang Preston Long,[†a] Mun Fei Eddy Wong,[a] Lee Ling Tan,[b] Elaine Tiong,[b] Fong Tian Wong,[b] Zhennan Liu*[a]

Laccase from *Trametes versicolor* was found to oxidize non-phenolic arenes and enable the trifluoromethylation of arenes in the presence of in situ generated-CF_3 radicals at a catalyst loading as low as 0.0034%. The biocatalytic trifluoromethylation proceeded under mild conditions and could increase the yield by up to 12 fold, as compared to the control.

Fluorinated compounds have found widespread applications across various fields of science and technology, such as medicinal chemistry, molecular imaging, and material science.[1-4] This is primarily due to the profound ability of fluorination to alter the physicochemical properties of small molecules and materials with minimum steric hinderance. In particular, the trifluoromethyl group represents a privileged unit among all the fluoroalkyl moieties and arenes bearing trifluoromethyl group constitute an abundant motif in agrochemicals and pharmaceuticals (Fig. 1).[5-7]

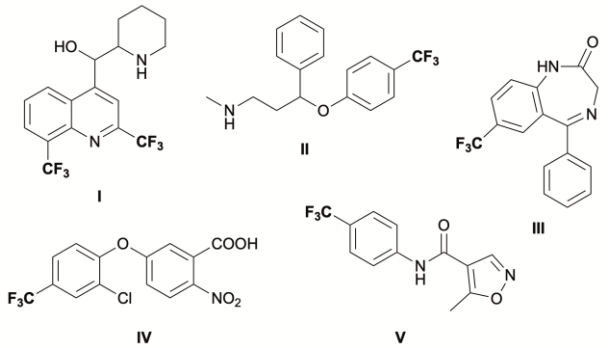


Figure 1. Representative examples of pharmaceutical and agrochemical compounds containing (trifluoromethyl)arenes. I: mefloquine; II: fluoxetine; III: triflunordazepam; IV: acifluorfen; V: leflunomide.

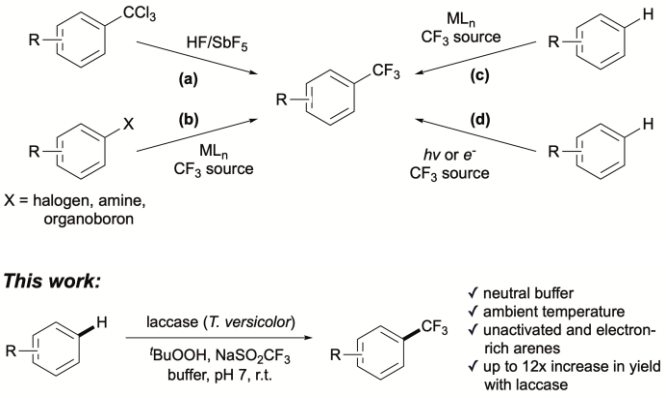


Figure 2. Methods for the trifluoromethylation of arenes.

On an industrial scale, the preparation of simple benzotrifluoride is typically achieved by fluorine–chlorine exchange of an aromatic–CCl_3 group to a trifluoromethyl group in the presence of SbF_5 or HF (Fig. 2a).[8] However, the low functional group tolerance, the use of hazardous reagents and the toxic waste generated, limit the generality of the approach. Therefore, the development of new and milder methodologies to create aryl-CF_3 bond holds enormous importance and attracts much attention from the research and industrial community.[5, 6, 9, 10] While transition metal-catalyzed cross-coupling reactions between pre-functionalized arenes and various CF_3 precursors have facilitated the introduction of CF_3 group in a specific manner (Fig. 2b),[11-16] direct trifluoromethylation of aromatic C-H bonds represents a straightforward approach with high step- and atom-economy to meet the industrial need of simplicity and cost efficiency for

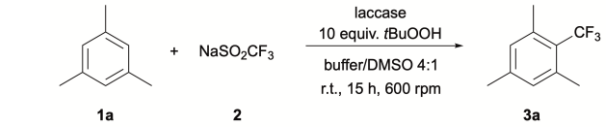
[a] *Institute of Sustainability for Chemicals, Energy and Environment (ISCE2), Agency for Science, Technology and Research (A*STAR), 8 Biomedical Grove, Neuros #07-01, Singapore 138665, Republic of Singapore.*
[b] *Institute of Molecular and Cell Biology (IMCB), Agency for Science, Technology and Research (A*STAR), 61 Biopolis Drive, Proteos #07-01, Singapore 138673, Republic of Singapore.*
† These authors contributed equally to this work.
Electronic Supplementary Information (ESI) available.

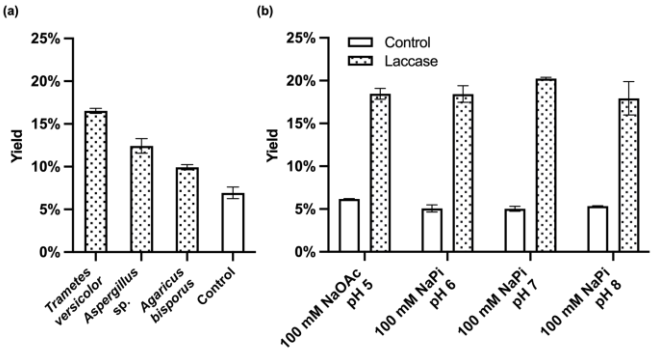
mass production. So far, two major strategies have been reported for direct trifluoromethylation of aromatic C-H bonds, one relying on a high-valent M(Ar)(CF_3) intermediate (M: transition metal; Ar: aryl ligand) to undergo reductive elimination to form Ar-CF_3 bond (Fig. 2c),[17-19] and the other based on the generation of free CF_3 radicals by light or peroxides to add to the arenes (Fig. 2d).[20-23]

Among different strategies reported for the radical introduction of CF_3 group onto arenes, one unique mechanism is to exploit the natural reactivity of laccases to catalyze one-electron oxidation of phenolic compounds to generate phenol-derived radicals.[24] In combination with the CF_3 radical generated in situ from either Langlois reagent ($NaSO_2CF_3$)[25] or Baran's zinc sulfinate ($Zn(SO_2CF_3)_2$),[26] the recombination of two radicals gave trifluoromethylated phenols. However, in this particular biocatalytic instance of radical trifluoromethylation, only a single laccase, from *Agaricus bisporus*, was examined, and the range of substrates was restricted to phenolic compounds that featured an electron-withdrawing group at the *para* position (such as ketones, aldehydes, esters, and nitriles).

In nature, laccases are produced by many different organisms and belong to a larger family of multicopper oxidases.[27-29] These enzymes can act upon a broad spectrum of substrates, in which the paramagnetic copper (type I) catalyzes monoelectronic oxidation of substrates in a cavity partially exposed to the surface and transfers the electron to a buried trinuclear copper center (type II/III). Laccases from different sources are found with a wide range of redox potentials, among which laccase from *Trametes versicolor* (*Tv*L) has been reported with one of the highest redox potential (785 mV versus the standard hydrogen electrode).[30] High-redox potential laccases are known to display high catalytic activity;[31, 32] therefore, we envisage that *Tv*L would have the capacity to oxidize non-phenolic aromatic compounds that cannot be effectively transformed by laccases with low or medium redox potentials. Herein we report a biocatalytic strategy for direct C-H trifluoromethylation of arenes by employing a high-redox potential fungal laccase from *T. versicolor*.

We first compared the catalytic activity of three commercially available fungal laccases (from *Trametes versicolor*, *Aspergillus sp.*, and *Agaricus bisporus*, respectively) for introduction of trifluoromethyl group onto a readily accessible aromatic substrate that contains unactivated C-H bonds, mesitylene (**1a**). By employing sodium triflinate (**2**) as trifluoromethylation reagent and *tert*-butyl hydroperoxide (*t*BuOOH) as the oxidant in a neutral buffer (100 mM NaPi, pH 7), we found that the reaction could proceed under a catalyst-free condition with a low yield of 6.9% (Fig. 3a). Upon addition of laccase, the yields could be increased up to 16.5%. Among the three laccases screened, the reaction catalyzed by *Tv*L afforded the mono-trifluoromethylated product with the highest yield, which corroborates our hypothesis that laccase with high redox potential could activate non-phenolic arenes with higher efficiency. We next evaluated the catalytic activity of *Tv*L under different pH conditions. It is known that most fungal laccases function optimally toward phenolic substrates at an acidic range of pH around 3.0-5.5,[33] but we found that *Tv*L catalyzed the trifluoromethylation of mesitylene with similar level of reactivity under different buffer conditions, with the reaction in the pH 7 buffer displaying a slightly higher yield of 20.2% than those occurring under other conditions (Fig. 3b). Other reaction parameters including the percentage of organic solvent and the equivalents of oxidants were also investigated. It was found that a lower amount of DMSO (<20%) in the reaction system decreased the reaction yield, presumably due to the low solubility of non-polar substrates without the assistance of co-solvents (Fig. S1). In addition, the amount of *t*BuOOH could be decreased to 5 equivalents without significant deterioration of the yield (Fig. S2).





(c)

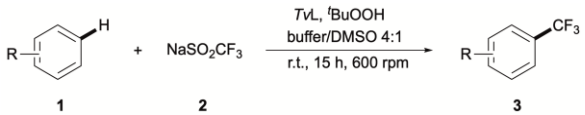
Entry	Catalyst	buffer	Conversion[b]	Yield[c]
1	laccase (*Tv*L)	100 mM NaPi, pH 7	68.1±1.1%	19.2±1.1%
2	desalted laccase[a]	100 mM NaPi, pH 7	68.9±0.8%	18.7±0.6%
3	-	100 mM NaPi, pH 7	56.9±4.3%	5.6±0.2%
4	-	ddH_2O	56.0±3.3%	6.2±0.1%

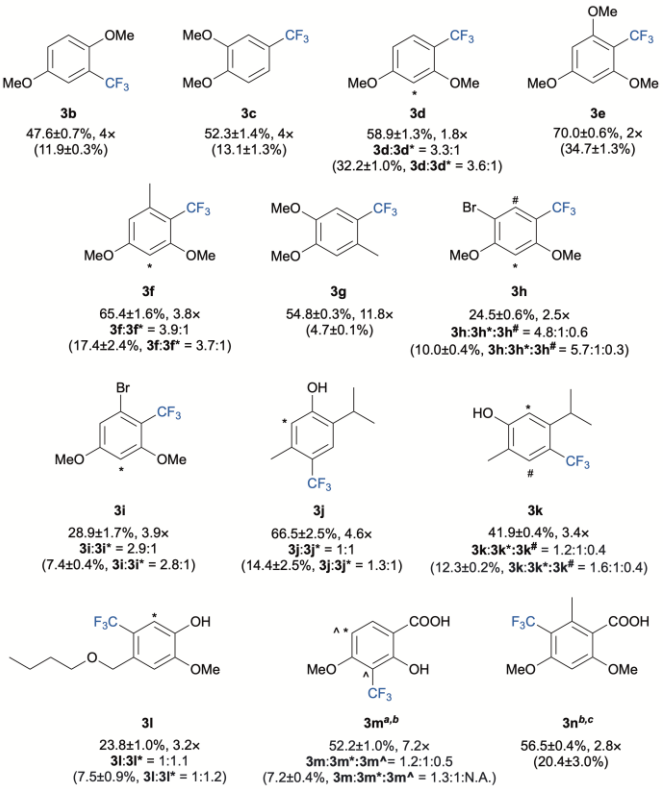
Figure 3. (a) Screening of trifluoromethylation reactivity of three commercially available laccases. (b) The reactivity of *Tv*L under different buffer conditions. (c) Comparison of the reactivity of commercial and desalted *Tv*L. Reaction conditions: mesitylene (**1a**, 0.02 mmol), $NaSO_2CF_3$ (5 equiv.), *t*BuOOH (10 equiv.), laccase (4 U), 20 vol.-% DMSO, NaPi buffer (100 mM, pH 7 or otherwise indicated), 25 °C, 600 rpm, 15 h. [a]*Tv*L desalted using a NAP-10 G-25 Sephadex column. [b]Determined by ^{1}H NMR with 2,2,2-trifluoroacetophenone as internal standard. [c]Determined by ^{19}F NMR with 2,2,2-trifluoroacetophenone as internal standard. Data shown are the average from three replicates, with error bars indicating 1 standard deviation.

Intriguingly, after the initial report of biocatalytic trifluoromethylation of phenols by laccase from *A. bisporus*,[24] a

follow-up study found that its trifluoromethylation activity was lost when desalted laccase was used in the reaction, and it was proposed that the unbound/dissociated copper ions in the biocatalyst solution were responsible for the trifluoromethylation reactivity.[8] In order to tell whether the enhancement of C-H trifluoromethylation reactivity stems from the free copper ions in the crude preparation of commercial laccase or from the laccase itself, a solution of *Tv*L was desalted by a Sephadex G-25 column and used directly for the reaction. We found that the desalted laccase catalyzed the C-H trifluoromethylation of mesitylene with the same level of reactivity as the un-desalted enzymes (18.7% vs. 19.2%, Fig. 3c), which indicates that the increase in the formation of trifluoromethylated product was attributed to *Tv*L rather than unbound metal ions possibly present in the commercial sample. ICP-MS analysis of commercial *Tv*L revealed that 1 U of laccase contains 0.68 nmol copper. As each active laccase contains 4 copper ions, the biocatalytic reaction was found to proceed with an extremely low catalyst loading of 0.0034%, denoting the high efficiency of enzymes (Table S1). A comparison of the catalytic activity of *Tv*L and inorganic copper salts ($Cu(NO_3)_2$) was conducted (Fig. S3), and we found that, under otherwise identical reaction conditions, *Tv*L (containing 7.0 μM Cu) catalyzed the trifluoromethylation of **1a** with 2.4 times the yield of the reaction catalyzed by 1000 μM $Cu(NO_3)_2$. This result implies that the catalytic activity of laccase is not solely attributed to the entrapment of copper ions but also to the enzyme scaffold.

The reaction was then extended to a range of arene substrates to evaluate the substrate scope of *Tv*L. As shown in Scheme 1, aromatics bearing electron-donating alkyl or alkoxyl groups with various substitution patterns could be transformed by the biocatalytic protocol to deliver the trifluoromethylated products with moderate to good yields (up to 70%, **3e**). Functional groups including methoxy (**3b-e**), methyl (**3f**,**g**), bromo (**3h**,**i**), hydroxyl (**3j**,**k**), long alkyl chain (**3l**), and carboxylic acid (**3m**,**n**), can be tolerated. Under catalyst-free conditions, we could observe a basal level of formation of trifluoromethylated arenes. With the addition of laccase, their yields were in general improved by 3-5 times. It is of note that *Tv*L could enhance the production of **3g** by nearly 12 fold. The different extents of improvement in yields are likely due to varying binding affinity of *Tv*L towards substrates. In terms of regioselectivity, C-H trifluoromethylation occurs preferentially at positions *ortho* or *para* to electron-donating groups and less sterically congested sites of the aryl rings, and similar level of regioselectivity was observed in both biocatalytic and control reactions.





Scheme 1. Direct C-H trifluoromethylation of arenes catalyzed by *Tv*L. Reaction conditions: arene (**1**, 0.02 mmol), $NaSO_2CF_3$ (5 equiv.), *t*BuOOH (10 equiv.), *Tv*L (4 U), 20 vol.-% DMSO, NaPi buffer (100 mM, pH 7), 25 °C, 600 rpm, 15 h. Control reactions were performed without laccase under otherwise identical conditions. Yields are determined by ^{19}F NMR with 2,2,2-trifluoroacetophenone as internal standard and yields of control reactions are shown in parentheses. Data shown are the average from three replicates. [a]Observed formation of bis-trifluoromethylated product **3m^**. [b]NaOAc buffer (250 mM, pH 5.4) was used. [c]Duplicate experiments.

Laccase is known to use oxygen as co-substrate and reduces it to water during the oxidation of substrates.[27-29, 33] To understand the role of laccase in this reaction, the oxidative trifluoromethylation of 1,3,5-trimethoxybenzene (**1e**) was conducted under both aerobic and anaerobic conditions with *Tv*L as catalyst, and similar level of yields were observed (Fig. S4a). In addition, by adding a radical scavenger, TEMPO (2,2,6,6-tetramethylpyperidinyloxy), the reaction was shut down completely (Fig. S4a). To further gain insights into the role of the C-H activation step, we synthesized a deuterated analog of **1e**. The kinetic isotope effect was evaluated by an intermolecular competition reaction between **1e** and **1e-D** and the isotopic product was obtained in a ratio of *ca* 1:1 (Fig. S4b). This KIE factor suggests that the C-H activation of arene is probably not involved in the rate-determining step.[34, 35]

Based on these mechanistic evidences, a putative mechanism was proposed (Fig. S5). In the absence of laccase, trace redox

metals in the buffer could activate *t*BuOOH to generate *t*BuO$^{\bullet}$ and OH^-. The *tert*-butoxy radical oxidizes triflinate anion ($CF_3SO_2^-$) to release SO_2 and $^{\bullet}CF_3$. The trifluoromethyl radical was captured by the arene substrate, generating the trifluoromethylated arene and another molecule of *t*BuO$^{\bullet}$ after re-oxidation by *t*BuOOH. In the presence of *Tv*L, the oxidoreductase couples the one-electron oxidation of electron-rich substrate to the reduction of *t*BuOOH. The arene radical cation thus generated recombines with the $^{\bullet}CF_3$ to form the C–CF_3 bond and yield the trifluoromethylated arene via re-aromatization. For substrates with high redox potentials, a similar mechanism as the background reaction may follow because the presence of laccase can possibly promote the generation of CF_3 radical in a higher concentration.

Conclusions

This method represents the first report of enzyme-dependent trifluoromethylation of non-phenolic aromatic compounds. Here, our studies proposed that fungal laccase from *Trametes versicolor*, with its high redox potential and substrate promiscuity, could oxidize non-phenolic arenes and generate arene radical cations that could recombine with trifluoromethyl radicals to form the trifluoromethylated product with enhanced yield. Further engineering of *Tv*L to increase its redox potential and substrate affinity is envisaged to improve the efficiency of the reaction.

Author Contributions

Goh Yi Ling: Investigation, Writing–Review and Editing; Shi Yang Preston Long: Methodology, Validation, Investigation; Mun Fei Eddy Wong: Methodology, Validation; Lee Ling Tan: Resources; Elaine Tiong: Resources; Fong Tian Wong: Writing–Review and Editing, Supervision, Funding acquisition; Zhennan Liu: Conceptualization, Methodology, Writing–Original Draft, Supervision, Funding acquisition.

Conflicts of interest

There are no conflicts to declare.

Acknowledgement

This research is supported by A*STAR Career Development Fund (C210812022). Shi Yang Preston Long was funded by A*STAR Research Internship Award.

Notes and references

1. M. Inoue, Y. Sumii and N. Shibata, *ACS Omega*, 2020, **5**, 10633-10640.
2. R. Berger, G. Resnati, P. Metrangolo, E. Weber and J. Hulliger, *Chem. Soc. Rev.*, 2011, **40**, 3496-3508.
3. G. Lewandowski, E. Meissner and E. Milchert, *J. Hazard. Mater.*, 2006, **136**, 385-391.
4. S. Purser, P. R. Moore, S. Swallow and V. Gouverneur, *Chem. Soc. Rev.*, 2008, **37**, 320-330.
5. A. Studer, *Angew. Chem. Int. Ed.*, 2012, **51**, 8950-8958.
6. X.-F. Wu, H. Neumann and M. Beller, *Chem. Asian J.*, 2012, **7**, 1744-1754.
7. J. Wang, M. Sánchez-Roselló, J. L. Aceña, C. del Pozo, A. E. Sorochinsky, S. Fustero, V. A. Soloshonok and H. Liu, *Chem. Rev.*, 2014, **114**, 2432-2506.
8. J. Pletz, C. Koeberl, M. Fuchs, O. Steiner, W. Goessler and W. Kroutil, *Eur. J. Org. Chem.*, 2019, **2019**, 682-690.
9. D. Mandal, S. Maji, T. Pal, S. K. Sinha and D. Maiti, *ChemComm*, 2022, **58**, 10442-10468.
10. T. Kliś, *Catalysts*, 2023, **13**, 94.
11. J.-J. Dai, C. Fang, B. Xiao, J. Yi, J. Xu, Z.-J. Liu, X. Lu, L. Liu and Y. Fu, *J. Am. Chem. Soc.*, 2013, **135**, 8436-8439.
12. X. Wang, Y. Xu, F. Mo, G. Ji, D. Qiu, J. Feng, Y. Ye, S. Zhang, Y. Zhang and J. Wang, *J. Am. Chem. Soc.*, 2013, **135**, 10330-10333.
13. G. Danoun, B. Bayarmagnai, M. F. Grünberg and L. J. Gooßen, *Angew. Chem. Int. Ed.*, 2013, **52**, 7972-7975.
14. M. Chen and S. L. Buchwald, *Angew. Chem. Int. Ed.*, 2013, **52**, 11628-11631.
15. E. J. Cho, T. D. Senecal, T. Kinzel, Y. Zhang, D. A. Watson and S. L. Buchwald, *Science*, 2010, **328**, 1679-1681.
16. H. Morimoto, T. Tsubogo, N. D. Litvinas and J. F. Hartwig, *Angew. Chem. Int. Ed.*, 2011, **50**, 3793-3798.
17. N. D. Ball, J. B. Gary, Y. Ye and M. S. Sanford, *J. Am. Chem. Soc.*, 2011, **133**, 7577-7584.
18. X.-G. Zhang, H.-X. Dai, M. Wasa and J.-Q. Yu, *J. Am. Chem. Soc.*, 2012, **134**, 11948-11951.
19. H. Sindhe, B. Chaudhary, N. Chowdhury, A. Kamble, V. Kumar, A. Lad and S. Sharma, *Org. Chem. Front.*, 2022, **9**, 1742-1775.
20. D. A. Nagib and D. W. C. MacMillan, *Nature*, 2011, **480**, 224-228.
21. Y. Ye, S. H. Lee and M. S. Sanford, *Org. Lett.*, 2011, **13**, 5464-5467.
22. J. W. Beatty, J. J. Douglas, K. P. Cole and C. R. J. Stephenson, *Nat. Commun.*, 2015, **6**, 7919.
23. N. Ichiishi, J. P. Caldwell, M. Lin, W. Zhong, X. Zhu, E. Streckfuss, H.-Y. Kim, C. A. Parish and S. W. Krska, *Chem. Sci.*, 2018, **9**, 4168-4175.
24. R. C. Simon, E. Busto, N. Richter, V. Resch, K. N. Houk and W. Kroutil, *Nat. Commun.*, 2016, **7**, 13323.
25. C. Wakselman, M. Tordeux, J.-L. Clavel and B. Langlois, *J. Chem. Soc., Chem. Commun.*, 1991, DOI: 10.1039/C39910000993, 993-994.
26. Y. Fujiwara, J. A. Dixon, F. O'Hara, E. D. Funder, D. D. Dixon, R. A. Rodriguez, R. D. Baxter, B. Herlé, N. Sach, M. R. Collins, Y. Ishihara and P. S. Baran, *Nature*, 2012, **492**, 95-99.
27. T. Brugnari, D. M. Braga, C. S. A. dos Santos, B. H. C. Torres, T. A. Modkovski, C. W. I. Haminiuk and G. M. Maciel, *Bioresour. Bioprocess.*, 2021, **8**, 131.
28. G. Janusz, A. Pawlik, U. Świderska-Burek, J. Polak, J. Sulej, A. Jarosz-Wilkołazka and A. Paszczyński, *Int. J. Mol. Sci.*, 2020, **21**, 966.
29. I. Bassanini, E. E. Ferrandi, S. Riva and D. Monti, *Catalysts*, 2021, **11**, 26.

30. S. Kurniawati and J. A. Nicell, *Bioresour. Technol.*, 2008, **99**, 7825-7834.
31. S. Barber-Zucker, I. Mateljak, M. Goldsmith, M. Kupervaser, M. Alcalde and S. J. Fleishman, *ACS Catal.*, 2022, **12**, 13164-13173.
32. I. Mateljak and M. Alcalde, *ACS Sustain. Chem. Eng.*, 2021, **9**, 9632-9637.
33. P. Baldrian, *FEMS Microbiol. Rev.*, 2006, **30**, 215-242.
34. M. Gómez-Gallego and M. A. Sierra, *Chem. Rev.*, 2011, **111**, 4857-4963.
35. E. M. Simmons and J. F. Hartwig, *Angew. Chem. Int. Ed.*, 2012, **51**, 3066-3072.