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# Acetamido-TEMPO mediated electrochemical oxidation of alcohols to aldehydes and ketones†

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A protocol for the oxidation of alcohols to aldehydes and ketones employing an electrochemical aminoxyl-mediated reaction is presented. The approach employs a catalytic amount of the radical and the use of a base is not required. It is performed using readily available electrodes in a commercially available electrochemistry apparatus and does not require a reference electrode. The methodology is applicable to a range of structurally and electronically diverse substrates, including the oxidation of primary alcohols to aldehydes rather than the more commonly formed carboxylic acids.

## Introduction

Using electricity as a tool for preparative organic chemistry has gained significant attention recently.<sup>1–4</sup> This is because electrochemistry offers an attractive alternative to conventional approaches to performing known functional-group transformations as well as opening the door for exploring new chemical space.<sup>5,6</sup> One area of particular interest is oxidation and reduction reactions where electrons can replace traditional oxidising or reducing agents that are often toxic, hazardous, or costly. As such, this makes electrochemistry attractive for both academic and industrial settings.<sup>7–10</sup>

Most approaches to synthesis using electrochemistry have employed “homemade” equipment. This can result in issues with reproducibility due to variations in reactor dimensions, electrode sources, and current density. However, with the advent of commercially available, specifically designed equipment, more standardised methodologies can be developed.<sup>11,12</sup>

With an eye to oxidation reactions, aminoxyl compounds such as 2,2,6,6-tetramethylpiperidinyloxy (TEMPO), 4-acetamido-TEMPO (ACT, **1**), and 9-azabicyclo[3.3.1]nonane *N*-oxyl (ABNO) have been explored as electrocatalysts.<sup>13–18</sup> These bench-stable radical species are easy to use, recoverable, and non-toxic, making them attractive from a sustainability standpoint.<sup>19–21</sup> Differing from direct electrolysis<sup>22</sup> where the electron-transfer (ET) process occurs on the electrode surface, electrocatalytic reactions use indirect electrolysis.<sup>7</sup> First, the electrocatalyst is activated by an ET on the electrode surface. It

then diffuses into the solution to react with the substrate in a homogeneous manner. The electrocatalyst is then regenerated, and the cycle repeats. This process allows reactions to be performed at lower potentials than anodic oxidation which broadens functional-group compatibility.

TEMPO, ACT, and ABNO have been employed by several groups as an electrocatalyst for the conversion of primary and secondary alcohols to aldehydes, ketones, or carboxylic acids (Fig. 1).<sup>23–27</sup> The published approaches use an excess of base and often employ expensive electrode materials such as platinum,<sup>23–28</sup> multi-walled carbon nanotubes (MWCNT),<sup>27</sup> or reticulated vitreous carbon (RVC)<sup>28</sup> to perform the transformations on what is often a narrow set of substrates, and expensive reference electrodes are sometimes required. A methodology for alcohol oxidation using an immobilized

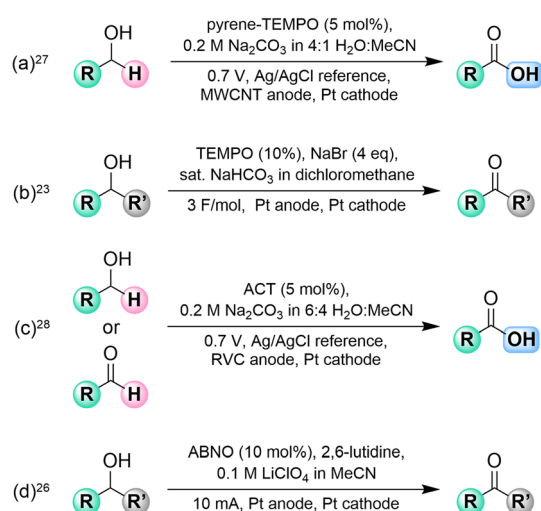


Fig. 1 Literature examples of nitroxide-mediated electrochemical oxidation.

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TEMPO catalyst has been developed (Fig. 1a)<sup>27</sup> as well as one for Anelli and Pinnick oxidations in basic media with a reference electrode (Fig. 1c).<sup>28</sup> Electrochemical oxidation of alcohols can also be achieved using TEMPO and a superstoichiometric quantity of sodium bromide (Fig. 1b)<sup>23</sup> or, in the case of secondary alcohols, by using ABNO as the electrocatalyst (Fig. 1d).<sup>26</sup>

Compared to other aminoxyl radicals, **1** is less costly and has been proven to operate well, if not better, at a comparable overpotential.<sup>14</sup> A case in point is the electrochemical oxidation of 5-hydroxymethylfurfural (HMF).<sup>29</sup> **1** also shows better electrocatalytic activity in the oxidation of alcohols compared with less hindered aminoxyls such as ABNO or 2-azaadamantane-*N*-oxyl (AZADO).<sup>30</sup> Despite this, the use of **1** as an electrocatalyst for oxidation reactions has not been extensively explored. When it is employed, primary alcohols are converted to carboxylic acids rather than aldehydes.<sup>28</sup>

Our group has significant experience using **1** in a variety of oxidation and oxidative functionalisation reactions, such as the transformation of alcohols and aldehydes to esters,<sup>31</sup> amides,<sup>32,33</sup> and nitriles.<sup>34,35</sup> Focusing on the oxidation of alcohols to aldehydes or ketones, we have used **1** in conjunction with visible-light photocatalysis (Fig. 2a).<sup>36</sup> Employing a dual catalytic system of Ru(bpy)<sub>3</sub>(PF<sub>6</sub>)<sub>2</sub> as a photocatalyst, **1** as the primary oxidant, and sodium persulfate as a terminal oxidant, the oxidation of a wide range of alcohols, including trifluoromethyl alcohols, is possible. More recently, we have developed an allied methodology employing only potassium persulfate and a catalytic amount of **1** under mild conditions (Fig. 2b).<sup>37</sup> However, the reactions still require superstoichiometric quantities of a terminal oxidant.

Spurred on by the possibility of substituting chemical oxidants with electrons, we envisioned an electrocatalytic approach for the oxidation of alcohols to aldehydes and ketones using **1** (Fig. 2c). We report the results from this study here. Highlights and novel aspects of our approach are that:

- the transformation is base-free;

- inexpensive electrode materials and a commercially-available electrochemistry unit are used, thereby ensuring reproducibility, and offering greater utility to the synthetic chemist;

- a simple two-electrode undivided cell is employed; there is no need for a reference electrode;

- the substrate scope of the methodology is diverse, including sensitive propargylic and  $\alpha$ -trifluoromethyl alcohols;

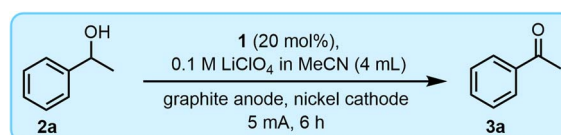
- the aldehyde product is generated, in the case of primary alcohols, without overoxidation to the corresponding carboxylic acid;

- product isolation is simple and does not require column chromatography.

## Results and discussion

As a starting point for developing reaction conditions for the electrochemical oxidation of alcohols to aldehydes and ketones using **1**, we decided to focus attention on the conversion of 1-phenylethanol (**2a**) to acetophenone (**3a**). When performing the reaction using 20 mol% of **1**, lithium perchlorate in acetonitrile (0.1 M, 4 mL) as the electrolyte and solvent, and a graphite anode and nickel cathode, we obtained a 54% conversion of **2a** to **3a** after 6 h at a constant current of 5 mA (Table 1, entry 1). The addition of base to the reaction mixture had no observable effect on product conversion, thereby showing that it is not a required component (entries 2 and 3). This is noteworthy

Table 1 Optimisation of reaction conditions<sup>ab</sup>



Entry	Change from conditions above	<b>3a</b> (%)
1	None	54%
2	Addition of 5 eq. pyridine	55%
3	Addition of 5 eq. 2,6-lutidine	53%
4	30 mol% <b>1</b>	55%
5	20 mol% TEMPO in place of <b>1</b>	52%
6	No <b>1</b> added	25%
7	10 mA	— <sup>c</sup>
8	No current	0%
9	0.1 M [TBA][PF <sub>6</sub> ] in place of LiClO <sub>4</sub>	20%
10	0.1 M [TBA][BF <sub>4</sub> ] in place of LiClO <sub>4</sub>	13%
11	0.1 M [TBA][ClO <sub>4</sub> ] in place of LiClO <sub>4</sub>	21%
12	0.1 M NaClO <sub>4</sub> in place of LiClO <sub>4</sub>	42%
13	0.5 mmol <b>2a</b> and reaction time of 3 h	43%
14	0.5 mmol <b>2a</b> and reaction time of 6 h	98%
15	2 mmol <b>2a</b> and reaction time of 24 h	99%
16	0.5 mmol <b>2a</b> , <b>3h</b> , Pt anode, Ni cathode	46%
17	0.5 mmol <b>2a</b> , <b>3h</b> , glassy carbon anode, Ni cathode	41%
18	0.5 mmol <b>2a</b> , <b>3h</b> , graphite anode, Pt cathode	35%

<sup>a</sup> Reactions performed on the 1 mmol scale unless stated otherwise.

<sup>b</sup> Product conversion determined by <sup>1</sup>H-NMR spectroscopy.

<sup>c</sup> Significant electrode fouling observed.

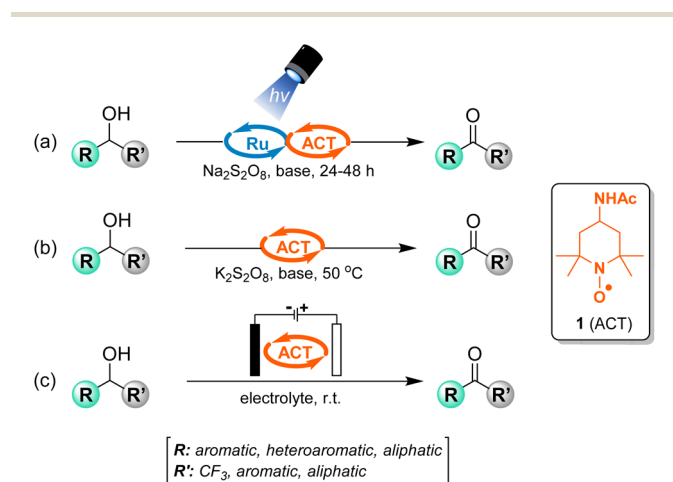


Fig. 2 Development of oxidation methodologies using ACT, **1**, as the primary oxidant.



given that previous electrocatalytic approaches to the oxidation of alcohols using either TEMPO or **1** have involved the use of a base in excess.<sup>23–25</sup>

Increasing the quantity of **1** from 20 mol% to 30 mol% did not improve the outcome of the oxidation reaction, and replacing **1** with TEMPO resulted in a lower product conversion, proving that the former is a better catalyst (entries 4 and 5). This is consistent with the knowledge that **1** is a more active electrocatalyst than TEMPO and other less sterically hindered aminoxyls like ABNO or AZADO.<sup>30</sup> In the absence of **1**, only a small amount of **3a** is observed, this occurring as a result of direct oxidation of the substrate, **2a**, on the surface of the anode (entry 6). In addition, the absence of **1** significantly increases resistance and hence the requisite voltage required to maintain the reaction at constant current. This indicates that **1** is essential for performing the reaction under mild conditions. Cyclic voltammetry also supports the assertion that **1** acts as mediator, it being oxidised at lower potential than the substrate and the process being chemically reversible (See ESI†).

Remaining focused on the electrochemical component of the reaction, we increased the current from 5 mA to 10 mA. This resulted in a concomitant increase in product conversion (entry 7), but was at the cost of substantial electrode fouling and so did not prove advantageous overall. When no current was applied, the reaction did not occur, thereby proving that the process is electrochemically mediated (entry 8). Next, we explored the effect of the electrolyte on the reaction by screening different ion pairs systematically. Three different tetrabutylammonium (TBA) salts were used to probe the effect of the anion (entries 9–11). The use of the perchlorate salt (entry 11) resulted in better performance than the hexafluorophosphate and tetrafluoroborate salts (entries 9 and 10). This could be related to the hydrogen-bond acceptor capacity of perchlorate,<sup>38</sup> suggesting that this anion is non-innocent and assist the oxidation process itself. Turning to the cation, using sodium or TBA perchlorate instead of the lithium congener reduced the effectiveness of the reaction (entries 11 and 12). Since it showed the best performance in the oxidation of **2a**, and because of the ease of separation from **3a** at the end of the reaction, lithium perchlorate remained the electrolyte of choice.

We wanted to probe the effect of substrate concentration on the effectiveness of the oxidation reaction. When reduced from 0.250 M to 0.125 M, a 43% conversion to **3a** was obtained after 3 h, and the reaction was essentially complete after 6 h (entries 13 and 14). When raised to 0.500 M, the reaction took 24 h to reach full conversion, indicating that the reaction time required is proportional to the quantity of starting material used.

Platinum, glassy carbon, and graphite are common electrodes used in preparative organic electrochemistry, so we screened various combinations of these (entries 16–18). In each case, product conversions were comparable. As a result, we opted to remain with our initial selection of a graphite anode and a nickel cathode because of their low cost and easy accessibility. Thus, our optimised conditions were: alcohol substrate (2 mmol), ACT (20 mol%), lithium perchlorate in acetonitrile (0.1 M, 4 mL), a graphite anode and a nickel cathode, constant current (5 mA), for 24–48 h.

With optimised conditions in hand, we screened a range of alcohol substrates to evaluate the scope of our methodology (Fig. 3). Different 1-phenylethanol derivatives, with substituents in *ortho*-, *meta*- and *para*-positions were successfully oxidised to the corresponding ketones in good to excellent yields (**3a–3j**). A representative propargylic benzylic alcohol was effectively oxidised in good yield (**3k**). This is noteworthy

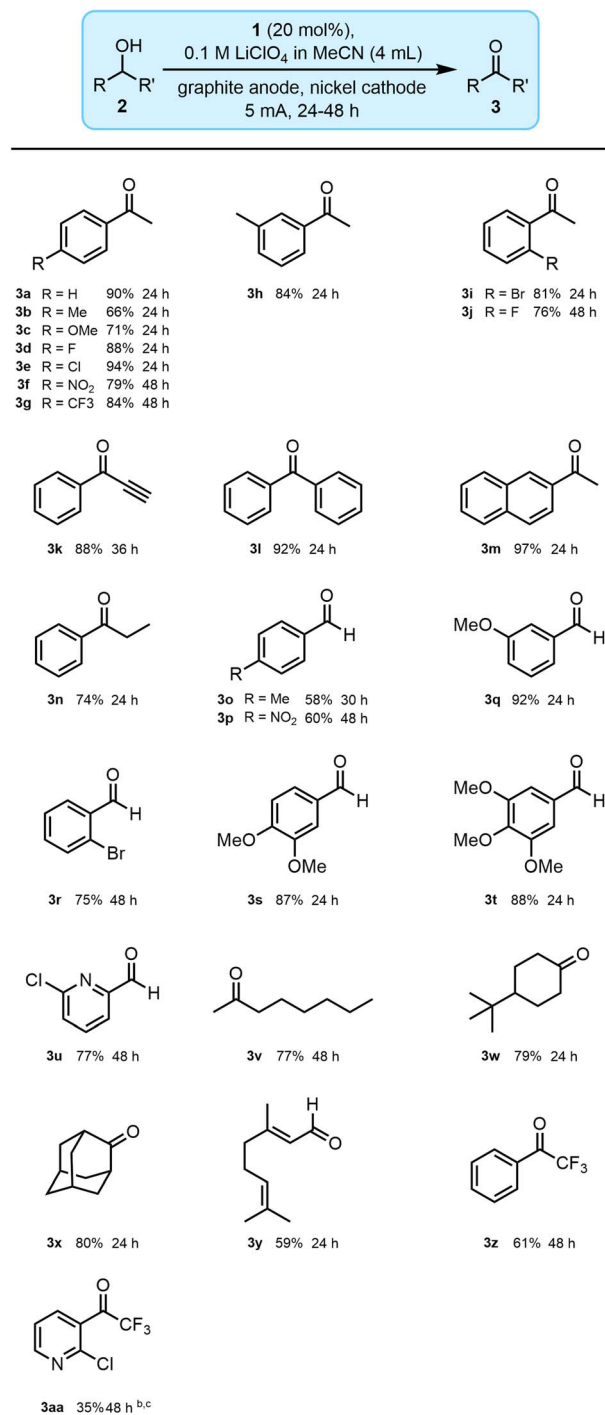


Fig. 3 Substrate scope for the oxidation of alcohols.<sup>a</sup> Performed on the 2 mmol scale; isolated yield after purification.<sup>b</sup> Performed on the 1 mmol scale.<sup>c</sup> Product conversion.



because it represents a class of sensitive substrates that typically decompose or generate off-target products when oxidised using conventional approaches.<sup>39</sup>

Biphenyl, naphthyl, and phenylpropyl alcohols could also be oxidised successfully to the ketones (**3l–3n**). Benzylic and heteroaromatic primary alcohols were oxidised to the corresponding aldehydes and not further transformed to carboxylic acids (**3o–3u**). Aliphatic alcohols were also amenable to the procedure (**3v–3x**), as was geraniol (**3y**). Two representative trifluoromethyl ketones (TFMK) were also prepared with conversions near 90% (**3z** and **3aa**). However, due to their low polarity, separating the desired TFMK product from the remaining  $\alpha$ -trifluoromethyl alcohol was not trivial and came at a cost to the isolated yield obtained. As is generally the case when using oxoammonium cations as oxidants, electron-deficient substrates require longer reaction times than their electron-rich congeners, as do steric materials bearing substituents in *ortho*-positions.<sup>21</sup> Due to its extreme electron-withdrawing nature of the substrate, **3aa** was synthesised on a smaller, 1 mmol scale to avoid a significantly extended reaction time. Overall, 27 representative alcohols were screened, with isolated yields of 58–97% being obtained.

A proposed mechanism for the oxidation process is shown in Fig. 4. The first step is the electrochemical generation of oxoammonium cation **4** from **1**. This takes place at the anode through a single electron transfer (SET) process. Facilitated by the perchlorate anion from the electrolyte, **4** then oxidises the alcohol substrate to form the carbonyl product. The hydroxylamine species, **5**, generated from this reaction is converted back into **1** at the anode by another SET process, thereby completing the catalytic cycle. At the cathode, the proton released from the alcohol oxidation step is reduced to H<sub>2</sub> (hydrogen evolution reaction).<sup>18,26–28,40</sup> This is supported by the observation of bubbles formed on the cathode surface over the course of the reaction.

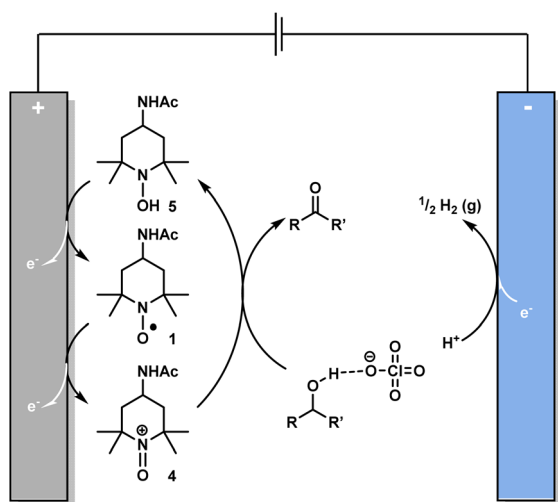


Fig. 4 Proposed mechanism for the electrochemical oxidation of alcohols.

## Conclusion

We have developed a methodology for the nitroxide-mediated electrochemical oxidation of alcohols to aldehydes and ketones. The reaction is performed in the absence of base, this representing an advance in terms of ease of use compared to similar procedures. Our approach also allows for the selective oxidation of primary alcohols to aldehydes rather than the corresponding carboxylic acids that are traditionally formed when using **1** as an electrocatalyst. The methodology uses inexpensive, widely available electrode materials, a simple electrolyte, and a small quantity of solvent. It is performed using a commercially available electrochemistry unit, making it reliable and reproducible. The approach proves effective for the oxidation of a range of aromatic, heteroaromatic, and aliphatic alcohols and can be applied to sensitive propargylic alcohols and to  $\alpha$ -trifluoromethyl alcohols, which are classes of compounds that are notoriously hard to oxidise.

## Experimental

### General considerations

NMR spectra (<sup>1</sup>H-, <sup>13</sup>C-, and <sup>19</sup>F-) were performed at 300 K using either a Bruker Avance Ultra Shield 300 MHz, or Bruker DRX-400 400 MHz spectrometer. <sup>1</sup>H-NMR spectra were referenced to residual CHCl<sub>3</sub> (7.26 ppm) in CDCl<sub>3</sub>. <sup>13</sup>C-NMR spectra were referenced to CDCl<sub>3</sub> (77.16 ppm). <sup>19</sup>F-NMR spectra were referenced to hexafluorobenzene (−161.64 ppm).<sup>41</sup> Reactions were monitored by <sup>1</sup>H- or <sup>19</sup>F-NMR, and/or by TLC on silica gel plates (60 Å porosity, 250 μm thickness). TLC plates were visualised using UV light. Electrochemistry reactions were performed using an IKA ElectraSyn 2.0 single vial holder and carousel units.

### Chemicals

Deuterated chloroform (CDCl<sub>3</sub>) was purchased from Cambridge Isotope Laboratories. Hexafluorobenzene was purchased from Oakwood Chemicals. 4-Acetamido-TEMPO (ACT, **1**) was prepared and recrystallised using a previously reported protocol.<sup>42</sup> All the alcohols used were purchased from Oakwood Chemicals, Sigma-Aldrich, or Alfa Aesar.

### General procedure for the preparation of ketones and aldehydes

Without precautions to exclude air or moisture; alcohol (2 mmol, 1 eq.), **1** (0.4 mmol, 0.2 eq.), lithium perchlorate in acetonitrile (4 mL, 0.1 M), and a stir bar were placed in an ElectraSyn 2.0 vial (5 mL capacity). The ElectraSyn vial cap was equipped with a graphite anode (working electrode) and a nickel cathode (counter electrode) and then secured onto the vial. The reaction mixture was stirred at 500 rpm. A constant current of 5 mA was passed through the reaction mixture until the oxidation was deemed complete, as monitored by <sup>1</sup>H-NMR spectroscopy (24–48 h). On completion, the product mixture was filtered through a pad of silica. The silica was washed with diethyl ether to elute the product and then the organic solvent



removed from the filtrate under vacuum to afford the pure product.

## Conflicts of interest

There are no conflicts to declare.

## Acknowledgements

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## Notes and references

- 1 H. Kolbe, *Ann. Chem. Pharm.*, 1849, **69**, 257–294.
- 2 M. M. Baizer, *Tetrahedron Lett.*, 1963, 973–977.
- 3 M. M. Baizer, in *Electrochemistry, Past and Present*, American Chemical Society, 1989, vol. 390, pp. 172–175.
- 4 J. Yoshida, K. Kataoka, R. Horcajada and A. Nagaki, *Chem. Rev.*, 2008, **108**, 2265–2299.
- 5 T. Gieshoff, S. R. Waldvogel, M. Zirbes, E. Rodrigo, A. Wiebe and S. Möhle, *Angew. Chem., Int. Ed.*, 2018, **57**, 5594–5619.
- 6 M. Yan, Y. Kawamata and P. S. Baran, *Chem. Rev.*, 2017, **117**, 13230–13319.
- 7 R. Francke and R. D. Little, *Chem. Soc. Rev.*, 2014, **43**, 2492–2521.
- 8 B. A. Frontana-Urbe, R. D. Little, J. G. Ibanez, A. Palma and R. Vasquez-Medrano, *Green Chem.*, 2010, **12**, 2099–2119.
- 9 D. S. P. Cardoso, B. Šljukić, D. M. F. Santos and C. A. C. Sequeira, *Org. Process Res. Dev.*, 2017, **21**, 1213–1226.
- 10 E. J. Horn, B. R. Rosen and P. S. Baran, *ACS Cent. Sci.*, 2016, **2**, 302–308.
- 11 M. Yan, Y. Kawamata and P. S. Baran, *Angew. Chem., Int. Ed.*, 2018, **57**, 4149–4155.
- 12 S. B. Beil, D. Pollok and S. R. Waldvogel, *Angew. Chem., Int. Ed.*, 2021, **60**, 14750–14759.
- 13 J. E. Nutting, M. Rafiee and S. S. Stahl, *Chem. Rev.*, 2018, **118**, 4834–4885.
- 14 F. Wang and S. S. Stahl, *Acc. Chem. Res.*, 2020, **53**, 561–574.
- 15 J. B. Gerken, Y. Q. Pang, M. B. Lauber and S. S. Stahl, *J. Org. Chem.*, 2018, **83**, 7323–7330.
- 16 R. Ciriminna, M. Ghahremani, B. Karimi and M. Pagliaro, *ChemistryOpen*, 2017, **6**, 5–10.
- 17 H. Hou, X. Ma, Y. Ye, M. Wu, S. Shi, W. Zheng, M. Lin, W. Sun and F. Ke, *RSC Adv.*, 2022, **12**, 5483–5488.
- 18 B. Karimi, M. Ghahremani, R. Ciriminna and M. Pagliaro, *Org. Process Res. Dev.*, 2018, **22**, 1298–1305.
- 19 I. B. Krylov, A. S. Budnikov, E. R. Lopat'eva, O. O. Segida, S. A. Paveliev and A. O. Terent'ev, *J. Phys.: Conf. Ser.*, 2021, **1942**, 012007.
- 20 H. A. Beejapur, Q. Zhang, K. Hu, L. Zhu, J. Wang and Z. Ye, *ACS Catal.*, 2019, **9**, 2777–2830.
- 21 J. M. Bobbitt, C. Brückner and N. Merbouh, in *Organic Reactions*, John Wiley & Sons, Ltd, 2010, pp. 103–424.
- 22 D. Wang, P. Wang, S. Wang, Y. H. Chen, H. Zhang and A. Lei, *Nat. Commun.*, 2019, **10**, 1–8.
- 23 Y. Demizu, H. Shiigi, T. Oda, Y. Matsumura and O. Onomura, *Tetrahedron Lett.*, 2008, **49**, 48–52.
- 24 M. Okimoto, T. Yoshida, M. Hoshi, T. Chiba and K. Maeo, *Synth. Commun.*, 2011, **41**, 3134–3139.
- 25 M. F. Semmelhack, C. S. Chou and D. A. Cortes, *J. Am. Chem. Soc.*, 1983, **105**, 4492–4494.
- 26 P. Niu, X. Liu, Z. Shen and M. Li, *Molecules*, 2019, **24**, 100.
- 27 A. Das and S. S. Stahl, *Angew. Chem., Int. Ed.*, 2017, **56**, 8892–8897.
- 28 M. Rafiee, Z. M. Konz, M. D. Graaf, H. F. Koolman and S. S. Stahl, *ACS Catal.*, 2018, **8**, 6738–6744.
- 29 A. C. Cardiel, B. J. Taitt and K.-S. Choi, *ACS Sustain. Chem. Eng.*, 2019, **7**, 11138–11149.
- 30 M. Rafiee, K. C. Miles and S. S. Stahl, *J. Am. Chem. Soc.*, 2015, **137**, 14751–14757.
- 31 A. León Sandoval, F. Politano, M. L. Witko and N. E. Leadbeater, *Org. Biomol. Chem.*, 2021, **19**, 2986–2990.
- 32 J. Nandi, M. Z. Vaughan, A. L. Sandoval, J. M. Paolillo and N. E. Leadbeater, *J. Org. Chem.*, 2020, **85**, 9219–9229.
- 33 F. Politano, A. León Sandoval, M. L. Witko, K. E. Doherty, C. M. Schroeder and N. E. Leadbeater, *Eur. J. Org. Chem.*, 2022, **2022**, e202101239.
- 34 J. Nandi and N. E. Leadbeater, *Org. Biomol. Chem.*, 2019, **17**, 9182–9186.
- 35 A. León Sandoval, F. Politano, M. L. Witko and N. E. Leadbeater, *Org. Biomol. Chem.*, 2022, **20**, 667–671.
- 36 V. A. Pistrutto, J. M. Paolillo, K. A. Bisset and N. E. Leadbeater, *Org. Biomol. Chem.*, 2018, **16**, 4715–4719.
- 37 F. Politano, W. P. Brydon and N. E. Leadbeater, *Synthesis*, 2023, **55**, 1517–1524.
- 38 S. J. Pike, J. J. Hutchinson and C. A. Hunter, *J. Am. Chem. Soc.*, 2017, **139**, 6700–6706.
- 39 C. E. Hatch, M. I. Martin, P. H. Gilmartin, L. Xiong, D. J. Beam, G. P. A. Yap, M. J. Von Bargen, J. Rosenthal and W. J. Chain, *Org. Lett.*, 2022, **24**, 1423–1428.
- 40 A. Badalyan and S. Stahl, *Nature*, 2016, **535**, 406–410.
- 41 C. P. Rosenau, B. J. Jellicoe, A. D. Gossert and A. Togni, *Angew. Chem., Int. Ed.*, 2018, **57**, 9528–9533.
- 42 M. A. Mercadante, C. B. Kelly, J. M. Bobbitt, L. J. Tilley and N. E. Leadbeater, *Nat. Protoc.*, 2013, **8**, 666–676.

