Electrochemical Synthesis of Isoxazolines: Method and Mechanism

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ABSTRACT: An electrochemical method for the green and practical synthesis of a broad range of substituted isoxazoline cores is presented. Both aryl and more challenging alkyl aldoximes are converted to the desired isoxazoline via an electrochemically enabled regio- and diastereoselective reaction with electron-deficient alkenes. Additionally, in-situ reaction monitoring methods compatible with electrochemistry equipment have also been developed in order to probe the reaction pathway. Supporting analyses from kinetic (time-course) modeling and density functional theory support a stepwise, radical-mediated mechanism, and discounts hypothesized involvement of closed shell [3+2] cycloaddition pathways.

Introduction
Isoxazolines, their derivatives, and related N,O-heterocycles are among the most important structural motifs in natural products.1,2 They possess many properties of value to the pharmaceutical industry, including antifungal and antibacterial activities.3 In particular, isoxazoline-containing natural products have received significant interest for their potential to exhibit potent anticancer activity (1 and 2, Figure 1a).4–7

Figure 1. Examples of isoxazoline-containing natural products and marketed drugs and pesticides.

Beyond natural products, isoxazolines are found to be important pharmacophores and can be seen in several marketed drugs, pesticides, and insecticides. Isoxazoline-containing drugs include Fluralener (3), an oral insecticide and acaricide for use in canine flea removal, and Topramezone (4), a pesticide that is currently used and marketed in the UK (Figure 1b). However, it is not only their biological properties that make these heterocycles desirable to the chemistry community, but also their use as masked structural motifs (Scheme 1).8,9 Much attention has therefore been paid to the synthesis of substituted isoxazoles and isoxazolines.

Scheme 1. Isoxazolines and Isoxazoles as Masked Structural Motifs.

Isoxazolines can be prepared from corresponding oximes via several methods, the most common of which is cyclization of a nitro oxide with a dipolarophile in a 1,3-dipolar cycloaddition (1,3-DC) reaction. Formation of nitro oxides are most commonly achieved in two ways: halogenation of oximes using electrophilic sources of halogen and subsequent base-promoted loss of HX (Scheme 2, Route A); or dehydration of nitroalkanes.10,11 Electrophilic chlorination of oximes has been the most explored method of in-situ preparation of hydroxymoyl...
Table 1. Optimization of Electrochemically Enabled 1,3-Dipolar Cycloaddition of Aldoxime 15a with Alkene 16a

| Entry | Electrode A:C | Mediator | Solvent | Additive (eq.) | F/mol-1 | Ratio17a:18 | Yield (17a+18)/% |
|-------|---------------|----------|---------|---------------|---------|-------------|------------------|
| 1     | RVC:RVC      | NaCl     | MeOH    | -             | 4.5     | -           | 31               |
| 2     | G:SS         | NaCl     | MeOH    | -             | 4.5     | 2:1         | 49               |
| 3     | G:SS         | NaI      | MeOH    | -             | 4.5     | 1.5:1       | 57               |
| 4     | G:SS         | Et4NCl   | MeOH    | -             | 4.5     | 1:1         | 48               |
| 5     | G:SS         | Et4NCl   | MeOH    | -             | 3       | 7:1         | 63               |
| 6     | G:SS         | Et4NCl   | MeOH    | -             | 5       | 6:1         | 39               |
| 7     | G:SS         | Et4NCl   | MeCN    | MeOH (34)     | 3       | 22:1        | 45               |
| 8     | G:SS         | Et4NCl   | HFIP    | -             | 3       | -           | 36               |
| 9     | G:SS         | Et4NCl   | MeCN    | HFIP (1.3)    | 3       | -           | 78               |
| 10    | G:SS         | Et4NCl   | MeCN    | HFIP (0.1)    | 3       | -           | 49               |
| 11    | G:SS         | Et4NCl   | MeCN    | HFIP (1.3)    | 5       | -           | 73               |
| 12    | G:SS         | Et4NCl   | MeCN    | TFE (1.3)     | 3       | -           | 74               |
| 13    | G:SS         | Et4NCl   | MeCN    | IPA (1.3)     | 3       | -           | 41               |
| 14    | G:SS         | Et4NCl   | MeCN    | HFIP (1.3)    | 0       | -           | 0                |

1Conditions: 15a (0.5 mmol), 16a (5 eq.), mediator (0.5 eq.), additive (0.5 eq.), solvent (7 mL), anode/cathode, 25 mA, [CT] F/mol-1.

2Ratio determined from isolated yields of products. 1Isolated yield. A = anode; C = cathode; RVC = reticulated vitreous carbon, G = graphite; SS = stainless steel; HFIP = 1,1,1,3,3,3-hexafluoroisopropanol; TFE = 1,1,1-trifluoroethanol; IPA = isopropanol.

halides 9 and 10 (X = Cl and Br, respectively), which are precursors to nitrite oxides 11.12-15 It is well known that nitrite oxides are highly reactive intermediates and can dimerize rapidly to form furanox 13.16 and alkyl nitrite oxides are most notorious for this undesired dimerization event.17,18 We envisaged that the competing dimerization reaction could be minimized by establishing a controlled electrochemical synthesis of isoxazolines wherein concentration of the intermediate nitrite oxide dipole 11 was kept strategically low versus the reaction partner 12 (Scheme 2, Route B).

Scheme 2. Rationale for electrochemical route to in-situ preparations of nitrite oxide intermediates towards isoxazolines.

Electro-organic synthesis has recently reemerged as a thriving field of synthetic chemistry due, in part, to the drive for reactions with improved environmental profiles.19-23 Treating electrons as reagents and electrodes as reactants that are not consumed during the course of the reaction, electrochemistry holds promise in being able to comply with the 12 principles of green chemistry,24 as well as safety, environmental, legal, economic, control, and throughput (S.E.L.E.C.T.) criteria for process scale-up towards manufacture.25 Electrochemistry can also circumvent the use of strong oxidizing or reducing agents, avoiding toxic waste generation. Additionally, the use of electrons as “catalysts26 in redox transformations can offer a powerful design strategy versus synthetic methods based on traditional catalysts/reagents.

Electrochemistry on an industrial scale has been used for many years, particularly in the refining of ores. Aluminum metal is refined by electrolysis of its molten ore at a carbon electrode and produces metric tons of aluminum each year.27 As an exemplar of a scaled synthetic electro-organic reaction, adiponitrile is produced on a large scale from the electrochemical dimerization of acrylonitrile and is used in the production of nylon-6,6.28 However, in the pharmaceutical industry, electrochemistry is in the very earliest stages of adoption. Part of the barrier to broad adoption of electrochemical synthesis can be attributed to some extent to the lack standardized enabling technologies.19 Prior to IKA’s release of the ElectraSyn 2.0,29,30 electrochemistry required bespoke glassware and equipment and, in terms of necessary entry-level expertise, represented a barrier to broad uptake of the electrochemical cell as a powerful synthesis-enabling tool.

Results and Discussion

Reaction Optimization. Building on previous work from Shono,31 it was envisioned that the chlorination of oximes could be achieved through the electrochemical oxidation of chloride anions to an electrophilic chlorinating species. In-situ generation of the nitrite oxide and 1,3-dipolar cycloaddition (DC) with
a dipolarophile partner could occur, furnishing the desired substituted isoxazolines. This hypothesis served as the basis for our method development and was only challenged during later mechanistic studies.

Benzaldehyde oxime 15a and tert-butyl acrylate 16a were chosen as model substrates for the exploration of our electrochemically enabled 1,3-DC reaction, with initial conditions of reticulated vitreous carbon (RVC) as both anode and cathode, NaCl as mediator, and a charge transfer of 4.5 F mol⁻¹ at a current of 25 mA in MeOH (Table 1, Entry 1). As shown in Entry 1, the initial conditions provided isoxazoline 17a in 31% isolated yield. The methyl (rather than tert-butyl) product of 17a was also observed, with the assumption that the methanolic solvent is non-innocent. Changing the electrode materials to a graphite (G) anode and stainless steel (SS) cathode, gave an increase in yield, but with a poorer ratio of desired : undesired products (Entry 2 vs. Entry 1). NaI as mediator in place of NaCl also gave an increase in yield, with a more favorable ratio of products (Entry 3 vs. Entry 2). The use of Et₄N⁺Cl⁻ as mediator and electrolyte gave a reaction profile that evidenced negligible quantities of observable impurities, with only a small decrease in yield (Entry 4). The improved solubility profile of a tetraalkylammonium salt as mediator allowed the exploration of other solvents, potentially eliminating the undesired transesterification of the target isoxazoline. To this end, reducing the charge transferred (Entry 5) and switching the solvent to MeCN with 1.3 eq. of HFIP (Entry 9) gave notably improved yields (63% and 78%, respectively) and no observable side-products (by LCMS or ¹H NMR). HFIP has been shown to possess properties that can stabilize radical and reactive intermediates. Interestingly, the reaction proceeded, albeit less efficiently, with only 10 mol% of HFIP in MeCN (Entry 10 vs. Entry 9). Transferring more than 3 F mol⁻¹ of charge (Entry 11) and using TFE instead of HFIP (Entry 12) furnished the desired isoxazoline in only slightly lower yields (73% and 74% vs. 78%). As was expected, in the presence of no electricity, no reaction was observed (Entry 14). Full optimization tables and preparative details can be found in the ESL.

**Aldoxime Scope.** With optimized conditions in hand, attention was turned to a substrate scope in the oxime partner. Both (at least presumably) electron-rich (forming products 17b-17c, and 17x-17z; Scheme 3) and electron-poor (17d-17w) benzaldehyde oximes were well tolerated, with moderate to good yields achieved. Interestingly, the substitution on the phenyl ring had a marked effect on the yield of the reaction; in general, meta-substitution gave the highest yield but with poorer observed regioselectivity. Furthermore, it was demonstrated that potentially electroactive groups such as I and Br were tolerated to a useful extent, with these moieties providing a chemical handle for further downstream chemistry (17d-17f and 17g-17i, respectively). Mesityl oxime was smoothly converted to the corresponding isoxazoline 17aa in 58% yield. Alkyl oximes were well tolerated with 17ab-17af isolated in good yields. The number of methylene units between the oxime functionality and the phenyl group has a large influence on the outcome of the reaction, with 17ab (one methylene spacer) isolated in 50% and 17ac (two methylene spacers) in 74% yield.

**Scheme 3. Scope of Electrochemically Enabled 1,3-Dipolar Cycloaddition of Aldoximes 15 and tert-Butyl Acrylate 16a**

| Oxime | Yield (Isolated) | Ratio | Conditions |
|-------|-----------------|-------|------------|
| 17a   | 76% (58%)       | 20:1  | Et₄N⁺Cl⁻/MeCN, 25 mA, 3 F mol⁻¹ |
| 17b   | 67%             | 25:1  | NaCl, MeCN, 30 mA |
| 17c   | 65%             | 25:1  | NaI, MeCN, 30 mA |
| 17d   | 70%             | 20:1  | Et₄N⁺Cl⁻/MeCN, 30 mA |
| 17e   | 57%             | 15:1  | NaCl, MeCN, 30 mA |
| 17f   | 60%             | 20:1  | NaI, MeCN, 30 mA |
| 17g   | 68%             | 16:1  | Et₄N⁺Cl⁻/MeCN, 30 mA |
| 17h   | 67%             | 25:1  | NaCl, MeCN, 30 mA |
| 17i   | 64%             | 25:1  | NaI, MeCN, 30 mA |
| 17j   | 72%             | 23:1  | Et₄N⁺Cl⁻/MeCN, 30 mA |
| 17k   | 70%             | 25:1  | NaCl, MeCN, 30 mA |
| 17l   | 70%             | 25:1  | NaI, MeCN, 30 mA |
| 17m   | 73%             | 16:1  | Et₄N⁺Cl⁻/MeCN, 30 mA |
| 17n   | 67%             | 20:1  | NaCl, MeCN, 30 mA |
| 17o   | 60%             | 20:1  | NaI, MeCN, 30 mA |
| 17p   | 70%             | 17:1  | Et₄N⁺Cl⁻/MeCN, 30 mA |
| 17q   | 59%             | 17:1  | NaCl, MeCN, 30 mA |
| 17r   | 65%             | 20:1  | NaI, MeCN, 30 mA |
| 17s   | 72%             | 23:1  | Et₄N⁺Cl⁻/MeCN, 30 mA |
| 17t   | 62%             | 20:1  | NaCl, MeCN, 30 mA |
| 17u   | 60%             | 20:1  | NaI, MeCN, 30 mA |
| 17v   | 52%             | 25:1  | Et₄N⁺Cl⁻/MeCN, 30 mA |
| 17w   | 74%             | 20:1  | NaCl, MeCN, 30 mA |
| 17x   | 64%             | 20:1  | NaI, MeCN, 30 mA |
| 17y   | 80%             | 14:1  | Et₄N⁺Cl⁻/MeCN, 30 mA |
| 17z   | 78%             | 20:1  | NaCl, MeCN, 30 mA |
| 17aa  | 58%             |       | Et₄N⁺Cl⁻/MeCN, 30 mA |
| 17ab  | 50%             |       | NaCl, MeCN, 30 mA |
| 17ac  | 74%             |       | NaI, MeCN, 30 mA |
| 17ad  | 86%             |       | Et₄N⁺Cl⁻/MeCN, 30 mA |
| 17ae  | 82%             |       | NaCl, MeCN, 30 mA |
| 17af  | 50%             |       | NaI, MeCN, 30 mA |
| 17ag  | 78%             |       | Et₄N⁺Cl⁻/MeCN, 30 mA |
| 17ah  | 34%             |       | NaCl, MeCN, 30 mA |
| 17ai  | 37%             |       | NaI, MeCN, 30 mA |

*Conditions: 15 (0.5 mmol), tert-butyl acrylate 16a (5 eq.), Et₄N⁺Cl⁻ (0.5 eq.), HFIP (1.3 eq.), MeCN (7 mL), G anode, SS cathode, 25 mA, 3 F mol⁻¹. Isolated yields. Regioisomeric ratios were determined by ¹H NMR of chromatographed products.*

Of note, cyclopropyl-substituted isoxazoline 17af was isolated in a serviceable 56% yield, suggesting that any radical intermediates generated under the reaction conditions can be used before intractable levels of competing side reactions that might otherwise ring open such strained functionality. Pyridyl aldoximes (17ag-17ai) were tolerated under the electrochemical reaction conditions, with no N-oxide or Minisci-type side products observed in the crude reaction mixture (by LCMS or ¹H NMR). Encouragingly, the model reaction also worked on a gram-scale, providing 17a in 58% isolated yield.
Scheme 4. Substrate Scope of Electrochemically Enabled 1,3-Dipolar Cycloaddition of (E)-Benzaldehyde Oxime 15a with Dipolarophiles 16

Dipolarophile Scope. Attention was then turned to scope in dipolarophile reaction partner (Scheme 4). Methyl acrylate and amides were tolerated, with the corresponding isoxazolines 18 and 20c-20e isolated in good yields. Pleasingly, medicinally relevant amide substituted isoxazoline 20c was obtained in 36% isolated yield. Acrylonitrile participated in the electrochemical reaction without incident, providing 20f in a good 77% isolated yield. 1,3-DC with disubstituted alkene dimethyl fumarate (trans-alkene) gave 20h in 59% with a diastereomeric of 9:1 in favor of the expected anti-diastereoisomer. However, when employing dimethyl maleate (cis-alkene) as the dipolarophile, the same diastereomeric ratio as observed for dimethyl fumarate (trans-alkene), 9:1 in favor of the anti-diastereoisomer, was observed. Pericyclic 1,3-DC reactions are known to proceed stereospecifically such that cis-alkene dipolarophiles would be expected to give rise to syn-diastereomeric isoxazolines, and not trans-isoxazolines, as observed with 20a.13 Through reaction monitoring and synthesis via non-electrochemical methods, it was observed that the expected syn-19a is chemically unstable and rapidly isomerizes to the more thermodynamically favorable anti-diastereomeric product. As such, the apparently stereoselective formation of trans-20a does not rule out a pericyclic 1,3-DC reaction mechanism (see mechanistic discussion below). Under the optimized conditions, styrene was not tolerated, however, upon switching the mediator to Et₃NI, solvent to MeOH and transferring a charge of 5 F mol⁻¹, the phenyl-substituted isoxazoline 20g was isolated in 32% yield. It is suspected that styril derived dipolarophiles are not well tolerated in this reaction due to their propensity to polymerize under electrochemical conditions. The limitations of using styrenes in this electrochemical method are further exemplified in the ESI, and discussed further in the Computational Studies section. The broad applicability of the methodology is exemplified by isolation of 20h and 20i in poor to moderate yields having employed vinylpyridines as dipolarophiles.

Process mass intensity (PMI) tool is an open access web tool from the ACS website which can be used to determine the efficiency and greenness of a given reaction.34 It can be shown that the electrochemical methodology described herein is significantly more process-friendly (PMI = 187) versus a comparable non-electrochemical reaction35 (PMI = 2167). Comparison was made on the reaction itself as detailed in the ESI (for this work) and the publication used as comparator,36 excluding chromatography which was identical in both cases in our hands. Further details of the use of the PMI calculator can be found in the ESI.

The utility of isoxazolines as masked structural motifs or medicinally relevant fragments is shown in Scheme 5. Using isoxazolines isolated from our emergent electrochemical method, reduction by LiAlH₄37 gave the amino diol 22 directly in 30%. Hydrogenation using iron powder and ammonium chloride gave the aldol product 23 in 13%.38 Furthermore, amide 24 was accessed via hydrolysis with LiOH and amide coupling39 with 4-amino-(N-Boc)piperazine in 40% over the two steps.

Scheme 5. Derivatization of Isoxazoline 17a Demonstrating Versatility as Masked Structural Motif

IR Monitoring of the Bulk Medium. In-situ reaction monitoring was employed to compare a non-electrochemical Oxone-mediated method for producing isoxazolines35 with our electrochemical method (Figures 2; blue points). In the non-electrochemical profile, an induction period for oxime consumption and isoxazoline generation was observed, consistent with the formation of an intermediate prior to product formation. Once all oxime was consumed, the rate of product formation increases to a maximum. In the electrochemical reaction (Figure 2; orange points), clear differences are observed. An initial fast rate of product formation slowed to a linear rate over the majority of the time-course. Oxime decay was immediately linear, with no induction period, and remained linear until the reaction
was near completion. This is consistent with the observed pseudo-zero order decay of oxime in the bulk. It is worth noting that the IR probe is only able to monitor the bulk solution. As the electrochemical reaction is surface-mediated, reactive intermediates are presumably present in low concentrations, below the detection limits of the IR device measuring the bulk medium. Further details on the methods used to monitor the electrochemical reactions – including methods compatible with the ElectraSyn 2.0 – are available in the ESI.

NMR Monitoring of the Bulk Medium. 1H NMR kinetic analysis enabled a more detailed kinetic analysis of the electrochemical isoxazoline synthesis. Using a range of substituted aldoximes, Hammett analysis of both the initial rates (at the point of switching electricity on) and the rate of the linear profiles after the initial phase are shown in Figure 3. The analysis evidenced a shallow inverse V-shaped plot (well within the arguably negligible range of a single order of magnitude rate difference) suggesting that there may be a change in the contributions from the elementary steps of the reaction upon going from electron-rich to electron-poor benzaldehyde oximes. Complementary Swain-Lupton analysis suggested a 62:38 split in the contributions of field (F) and resonance (R) effects, respectively. Mechanistic studies on related photochemical methods of making isoxazolines from aldoximes by Leonori et al. revealed a non-inverse V-shaped Hammett plot with rates again spanning less than one order of magnitude.30 In relation to our initial [3+2] cycloaddition hypothesis, and with nitrile oxides being ambiphilic dipole able to react through either HOMO donor–LUMO acceptor or LUMO acceptor–HOMO donor frontier molecular orbital interactions, the Hammett plots could suggest a change in dominant orbital interactions as the substituent on the oxime is varied.31

Kinetic isotope effects (KIEs) by independent rate experiments were assessed using deuterated aldoxime 15a and deuterated aldoxime 15a-d_1. A small normal KIE of 1.0 – 1.5 was obtained (see ESI for detailed reaction profiles and KIE calculations). The negligible KIE suggests that C-H bond-breaking (or bond-forming) does not feature in the rate-limiting process of the overall reaction. The small KIE is also reflected in the similar isolated yields from the electrochemical reactions, with 15a-d_1 furnishing 17a in 70% (vs. 78% for 15a, Scheme 6).

Figure 2. ReactIR time courses of non-electrochemical (blue) and electrochemical (orange) reactions between benzaldehyde oxime 15a and tert-butyl acrylate 16a to yield isoxazoline 17a. See ESI for full details of reaction conditions.

Figure 3. Hammett analysis of observed rates of oxime 15 decay (red) and isoxazoline 17 formation (blue). Data were analyzed from t_0 along the linear pseudo-zero order regime of 1H NMR time courses. Swain-Lupton analysis: F : R = 68 : 32.

Scheme 6. KIE Measurements.

Conditions: 15a or 15a-d_1 (0.5 mmol), 16a (5 eq.), Et_4NCl (0.5 eq.), HFIP (1.3 eq.), MeCN (7 mL), G anode, SS cathode, 25 mA, 3 F.mol^-1.

Cyclic Voltammetry and Potentiometric Data. Cyclic voltammetry is a powerful analytical tool for probing electrochemical reactions.32 All cyclic voltammograms (CVs) herein used the ferrocene/ferrocenium (Fc/Fc^+) redox couple as a reference voltammogram (Figure 6). The Et_4NCl additive gave a reversible redox couple with an E_1/2 = 0.734 V (vs. Fc/Fc^+, purple line). tert-Butyl acrylate 16a showed no oxidative behavior within the swept potential range (Figure 6, orange line). Interestingly, the CV of (E)-benzaldehyde oxime 15a shows three distinct irreversible oxidative events, all of which are single-electron oxidations. The oxidation potentials of the first two events are in close proximity to one another and also suggests that the rate of the electron transfer for the first step is substantially lower than that of the second step.

When analyzing the reaction mixture as a whole, only two distinct oxidations near those associated with 15a were observed. The oxidation of the chloride anion was also observable in the reaction mixture. There was also an appreciable decrease in the reductive wave of the chloride couple in the whole reaction versus measurements with the chloride alone (Figure 5, green versus purple lines near 0.5 V). These data are consistent with the oxidized chloride species being consumed during the reaction. However, there is not a complete loss of a reductive wave for the chloride, which supports a mechanism manifesting either partial or catalytic consumption of chloride.
In-depth analysis of CV scan rates versus current was carried out using various combinations of the reactants, additives, and solvents used in the isoxazoline synthesis. Using the Randles-Sevcik relationship, exclusively linear plots of the square root of scan rate ($\sqrt{v}$) versus output current ($i_p$) of the redox events obtained from the CV experiments were observed. These data remained consistent with the interpretation that all key reacting species diffused freely in solution, with no irreversible chemical adsorption onto the electrodes (exemplified in Figure 6). Complementary analysis of reaction kinetics versus stirrer speed confirmed that rate of isoxazoline formation was independent of stirrer speed at >200rpm (see ESI).

Having established the oxidation potentials of the separate components of the reaction, attention was turned to measuring the potential of the electrochemical reaction over time. This electro-kinetic measurement serves to contextualize the oxidation potentials evidenced in the CV data, and identify which oxidation events are likely to occur under the applied conditions. The results are displayed in Figure 7, with the calculated potential at the anode plotted versus time. The measured potential at the anode is approximately steady (as expected under constant current electrolysis), and is always greater than the measured oxidation potentials of all reaction components. These kinetic measurements support at least two mechanisms en route to isoxazoline product 17; one via mediated consumption of the oxime by an oxidized chlorine species, and another by direct oxime oxidation.

Additive Effects. Control experiments performed in the absence of chloride salt – thought to play a dual role of both mediator and electrolyte - showed that the reaction still occurred, albeit with a greatly diminished yield of 24% versus 78% (Scheme 7a). This result implied possible direct oxidation of the oxime at the anode in the absence of chloride. In this control experiment, Et$_3$NCl was replaced with a halide-free alternative electrolyte, Et$_3$NTOs. In a second control experiment, the reaction was shown to proceed less efficiently in the absence of the HFIP co-solvent additive (Scheme 7b). This observation suggested that HFIP acts to stabilize electrochemically-generated radical intermediates. The stabilizing role of HFIP is also supported by DFT calculations (see below).

Stereochemical Analysis. The use of 1,2-disubstituted alkenes as nominal dipolarophiles provides mechanistic insight on account of the observed reaction diastereoselectivity. From Scheme 4, product 20a, it was observed that the reaction was stereoselective (rather than stereospecific) when using $E$- and...
Z-alkenes dimethylfumarate and dimethylmaleate, respectively. Both alkenes selectively produced the anti- as opposed to the syn-diastereomer of the isoxazoline product. In monitoring the kinetics of this process via \(^1H\) NMR, it became clear that, for both alkene starting materials, the anti-diastereomer formed first, while the syn-diastereomer emerged later in the reaction time course (Scheme 8, bottom). Together with the electrochemical data (Figures 5 – 7), these data supported stepwise mechanism(s) of isoxazoline formation (Scheme 8, Path A) rather than the initially hypothesized [3+2] pericyclic mechanism (Scheme 8, Path B).

Computational Studies. To support our experiments, a computational mechanistic study was conducted. Using density functional theory (DFT), mechanisms were investigated with single point energy corrections at the M062X/Def2TZVP level of theory, using Truhlar’s SMD variation of the integral-equation-formalism polarizable continuum model (IEF-PCM) for acetonitrile. M062X/6-31+G(d,p) was used to obtain gas phase free energy corrections and optimized geometries. Transition states were characterized via a single negative vibrational frequency, and their connection to intermediates shown to be consistent with intrinsic reaction coordinate (IRC) calculations.

In relation to Scheme 8, viable transition states were found for both Path A (stepwise radical-mediated) and Path B (concerted [3+2] cycloaddition). All calculations were consistent with radical mechanism (Path A) and not the initial [3+2] mechanistic hypothesis (Path B). Across combinations of substituted oximes and mono-/1,2-disubstituted alkenes reaction partners used experimentally, the [3+2] cycloaddition was shown to consistently yield calculated barriers 2.5-fold higher than for the stepwise radical mechanism. Consistent with the observations of diastereoselectivity, calculations along the radical pathway suggested an approximately 25-fold rate enhancement for reaction via the E- over the Z-alkene (Scheme 9). Potential energy surface scans suggested a low barrier to interconversion of intermediates arising from hydroximoyl radical attack on either the E- or Z-alkene, supporting a diastereoselective process (see ESI). The emergence of the syn-diastereomer after formation of the anti-diastereomer is consistent with epimerization of the kinetic anti- to the thermodynamic syn-product (favored by approx. 2 kcal/mol).

Scheme 9. Representative DFT calculations supporting open-shell radical over closed shell [3+2] mechanism towards isoxazoline products.

Scheme 10. DFT calculations supporting open-shell radical towards isoxazoline products.
DFT analysis of the radical pathway was also consistent with the observed positive influence of HFIP and chloride additives. As a simplified model of oxime oxidation and H-atom abstraction, potential energy surface scans were performed to monitor energy changes as a function of hydroxyimoyl C–H bond length. Whilst similar bond length versus electronic energy profiles were evidenced for the neutral singlet oxime, the singlet oxime-HFIP dimer, and the oxime radical cation, a significant energy stabilization was shown for the oxime radical cation-HFIP dimer (Scheme 11, bottom left). This result supports the original hypothesis that HFIP primarily serves as a radical stabilizer, namely the oxime radical cation. Moreover, HFIP is known to be electrochemically stable through a large potential window.46

Towards an understanding of the role of chloride as mediator, we found viable transition states for chlorine-mediated H-atom abstraction relating to all four C–H bond scan structures shown at the top of Scheme 11. The lowest barrier from the preactivation complex (3.6 kcal/mol) was found for the neutral singlet oxime in the absence of HFIP. However, there was once again a significant HFIP stabilization effect for chlorine-mediated H-atom abstraction from the oxime radical cation (7.9 versus 12.9 kcal/mol; Scheme 11, blue versus red).

Scheme 11. Potential energy surface scans of hydroxyimoyl C–H bonds in different oxidation states with and without HFIP binding (bottom left), and barrier heights for chlorine radical-mediated H-atom abstraction (bottom right).

In line with the Hammett analysis from Figure 3, DFT analysis of the S_{0}2 and cyclization steps of the proposed radical mechanism show a dynamic oxime substituent dependence. As the para-substituent becomes more electron-donating (as quantified by Hammett \( \omega \)), the barrier to nucleophilic attack goes down, whilst the barrier to the secondary cyclisation step goes up. Though the S_{0}2 step varies little between 13.4 – 13.8 kcal/mol, cyclization appeared much more sensitive, with substituents causing a 7.0 – 10.5 kcal/mol spread in barriers. The latter DFT calculated step is primarily field (F) driven, and is consistent with the experimental Swain-Lupton analysis which inferred 68 : 32 weighting in favor of field of resonance contributions. See ESI for full details.

The chlorine radical-mediated H-atom abstraction step was calculated to have a large primary KIE of 5.2. The KIE result appeared to contradict the negligible overall KIE observed experimentally (Scheme 6). Nonetheless, the result fit with the proposed radical mechanism, assuming all steps in the bulk (off-electrode) are limited by the slow production of chlorine radical on the anode (see ESI for KIE data and below for additional kinetic analysis). In other words, the observed lack of any KIE could be a false negative result.

Proposed Mechanistic Pathway. Through the combination of experimental observations and computational support, the following mechanistic model (running counter to our initial hypothesis) is proposed. In Scheme 12, two contributing radical mechanisms are shown, with the major contributor on top. Chloride, from the EtNCl additive, is oxidized on the anode, generating fleetingly small concentrations of chlorine radical. The chlorine radical concentration is on the order of ca. 10^{-11} M and is consistent with our geometric model of spherical chloride monolayer coverage of the 5 exposed faces of the approximately cuboidal electrode (see ESI). The chlorine radical then participates in H-atom abstraction from the oxime, generating the nucleophilic hydroxyimoyl radical. The radical reacts with the Michael acceptor, forming the C–C bond of the isoxazoline product. Subsequent formal 5-exo-tet cyclisation and cathodic reduction reveals the final isoxazoline product. A secondary pathway involving direct oxidation of the oxime to its radical cation, followed by HFIP-stabilized and chloride-mediated HAT, is also proposed, accounting for all experimental observations and DFT support.

Scheme 12. Proposed mechanisms of electrochemical isoxazoline formation.

Using the COPASI microkinetic modelling software,47 simulation of the chloride-mediated mechanism delineated in Scheme 12, with the inclusion of competing hydroxyimoyl radical degradation step, proved an attractive fit to the experimentally-determined NMR data (Scheme 13).

Conclusions

In conclusion, we have developed a broadly applicable electrochemical methodology for the synthesis of substituted isoxazolines that is quantifiably greener than comparable non-electrochemical methods. The method enables short reaction times, minimal waste generation, and avoids use of toxic or expensive oxidizing reagents. Both aromatic and alkyl substrates are tolerated, and exemplar heteroaryl aldoxime reaction partners have been applied successfully. Mechanistic analysis supported a surface-mediated electrochemical reaction and a stepwise radical process. The proposed mechanism, which ran counter to our hypothesized design of a genuine pericyclic 1,3-dipolar cycloaddition towards the targeted isoxazolines, accounted for
observed substituent, additive, and deuterium isotope effects, as well as diastere- and regioselective reaction outcomes.

**Scheme 13.** Kinetic simulation of oxime decay and isoxazoline product formation. Dashed lines represent COPASI simulations of the proposed mechanism.

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ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Experimental procedures, spectroscopic data, and all relevant NMR spectra (PDF)

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**Author Contributions**

All authors have given approval to the final version of the manuscript.

**Notes**

The authors declare no competing financial interest.

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**REFERENCES**

(1) Kumar, K. A.; Govindaraju, M.; Renuka, N.; Kumar, G. V. Isoxazolines: An Insight to Their Synthesis and Diverse Applications. *J. Chem. Pharm. Res.* 2015, 7 (3), 250–257.

(2) Hu, F.; Szostak, M. Recent Developments in the Synthesis and Reactivity of Isoxazoles: Metal Catalysis and Beyond. *Adv.

(3) Synth. Catal. 2015, 357 (12), 2583–2614. https://doi.org/10.1002/sct.201500319.

(4) Welsch, M. E.; Snyder, S. A.; Stockwell, B. R. Privileged Scaffolds for Library Design and Drug Discovery. *Curr. Opin. Chem. Biol.* 2010, 14 (3), 347–361. https://doi.org/10.1016/j.cbpa.2010.02.018.

(5) Kaar, K.; Kumar, V.; Sharma, A. K.; Gupta, G. K. Isoxazoline Containing Natural Products as Anticancer Agents: A Review, *Eur. J. Med. Chem.* 2014, 77, 121–133. https://doi.org/10.1016/j.ejmech.2014.02.063.

(6) Abou-Shoer, M. I.; Shaala, L. A.; Youssef, D. T. A.; Badr, J. M.; Habib, A.-A. M. Bioactive Brominated Metabolites from the Red Sea Sponge Suberea Mollis. *J. Nat. Prod.* 2008, 71 (8), 1464–1467. https://doi.org/10.1021/np071001-2xu.

(7) Kalaitzis, J. A.; Levine, P. de A.; Hooper, J. N. A.; Quinn, R. J. Ianthesine E, a New Bromotyrosine-Derived Metabolite from the Great Barrier Reef Sponge Pseudoceratina Sp. *Nat. Prod. Res.* 2008, 22 (14), 1257–1263. https://doi.org/10.1080/14786410701763411.

(8) Ichiba, T.; Scheuer, P. J.; Kelly-Borges, M. Three Bromotyrosine Derivatives, One Terminating in an Unprecedented Diketocyclopentenylidene Enamine. *J. Org. Chem.* 1993, 58 (15), 4149–4150. https://doi.org/10.1021/jo00067a062.

(9) Bode, J. W.; Hachisu, Y.; Matsuura, T.; Suzuki, K. Facile Construction and Divergent Transformation of Polycyclic Isoxazoles: Direct Access to Polyketide Architectures. *Org. Lett.* 2003, 5 (4), 391–394. https://doi.org/10.1021/ol027283f.

(10) Mukaïyama, T.; Hoshino, T. The Reactions of Primary Nitroparaflins with Isoxanates1. *J. Am. Chem. Soc.* 1960, 82 (20), 5339–5342. https://doi.org/10.1021/ja01505a017.

(11) Basel, Y.; Hassner, A. An Improved Method for Preparation of Nitro Oxides from Nitroalkanes for In Situ Dipolar Cycloadditions. *Synthesis (Stuttg.).* 1997, 1997 (03), 309–312. https://doi.org/10.1055/s-1997-1181.

(12) Liu, K.-C.; Shelton, B. R.; Howe, R. K. A Particularly Convenient Preparation of Benzohydroximinoyl Chlorides (Nitrite Oxide Precursors). *J. Org. Chem.* 1980, 45 (19), 3916–3918. https://doi.org/10.1021/jo01037a039.

(13) Gi, H.-J.; Xiang, Y.; Schinazi, R. F.; Zhao, K. Synthesis of Dihydrooxazoloxide Nucleoside and Nucleotide Analogs. *J. Org. Chem.* 1997, 62 (1), 88–92. https://doi.org/10.1021/jo961779r.

(14) Lee, G. A. A Simplified Synthesis of Unsaturated Nitrogen-Heterocycles Using Nitrite Betaines. *Synthesis (Stuttg.).* 1982, 1982 (06), 508–509. https://doi.org/10.1055/s-1982-29860.

(15) Ye, Y.; Zheng, Y.; Xu, G.-Y.; Liu, L.-Z. Reaction of Nitro Oxides with Vinylphosphonate: A Facile, Regioselective Approach to 5-Phosphonyl-1,4,5-Dihydrooxazoles. *Heterot. Chem.* 2003, 14 (3), 254–257. https://doi.org/10.1002/hc.10136.

(16) Hashimoto, T.; Maruoka, K. Recent Advances of Catalytic Asymmetric 1,3-Dipolar Cycloadditions. *Chem. Rev.* 2015, 150 (11), 5751–5805. https://doi.org/10.1021/cr5007182.

(17) Yoshimura, A.; Middleton, K. R.; Toda, A. D.; Kastern, B. J.; Koski, S. R.; Maskaev, A. V.; Zhdankin, V. V. Hypervalent Iodine Catalyzed Generation of Nitro Oxides from Oximes and Their Cycloaddition with Alkenes or Alkynes. *Org. Lett.* 2013, 15 (15), 4010–4013. https://doi.org/10.1021/ol401818n.

(18) Yoshimura, A.; Zhu, C.; Middleton, K. R.; Toda, A. D.; Kastern, B. J.; Maskaev, A. V; Zhdankin, V. V. Hypoiodite Mediated Synthesis of Isoxazolines from Aldoximes and Alkenes Using Catalytic IK and Oxone as the Terminal Oxidant. *Chem. Commun.* 2013, 49 (42), https://doi.org/10.1039/c3cc41164h.

(19) Yan, M.; Kawamata, Y.; Baran, P. S. Synthetic Organic Electrochemical Methods Since 2000: On the Verge of a Renaissance. *Chem. Rev.* 2017, 117 (21), 13230–13319. https://doi.org/10.1021/acs.chemrev.7b00397.

(20) Moeller, K. D. Synthetic Applications of Anodic Electrochemistry. *Tetrahedron* 2000, 56 (49), 9527–9554. https://doi.org/10.1016/S0040-4020(00)00840-1.

(21) Yoshida, J.; Kataoka, K.; Horcajada, R.; Nagaki, A. Modern Strategies in Electroorganic Synthesis. *Chem. Rev.* 2008, 108 (7), 2265–2299. https://doi.org/10.1021/cr0680843.
(22) Francke, R.; Little, R. D. Redox Catalysis in Organic Electrosynthesis: Basic Principles and Recent Developments. Chem. Soc. Rev. 2014, 43 (8), 2492. https://doi.org/10.1039/c3cs60464k.

(23) Sperry, J. B.; Wright, D. L. The Application of Cathodic Reductions and Anodic Oxidations in the Synthesis of Complex Molecules. ChemElectroChem 2006, 35 (7), 605–621. https://doi.org/10.1002/celc.20050982w.

(24) Anastas, P. T.; Warner, J. C. Green Chemistry: Theory and Practice; Oxford University Press: New York, 1998.

(25) Butters, M.; Catterick, D.; Craig, A.; Curzon, A.; Dale, D.; Gillmore, A.; Green, S. P.; Marziano, I.; Sherlock, J.-P.; White, W. Critical Assessment of Pharmaceutical Processes: A Rationale for Changing the Synthetic Route. Chem. Rev. 2006, 106 (7), 3002–3027. https://doi.org/10.1021/cr050982w.

(26) Studer, A.; Curran, D. F. The Electron Is a Catalyst. Nat. Chem. 2014, 6, 765. https://doi.org/10.1038/nchem.2031.

(27) Grotheer, M.; Alkire, R.; Varjani, R.; Srinivasan, V.; Weidner, J. Industrial Electrolysis and Electrochemical Engineering. Electrochim. Acta Interface 2006, 15 (1), 52–54.

(28) Cardoso, D. S. P.; Sljukić, B.; Santos, D. M. F.; Sequeira, C. A. C. Organic Electrosynthesis: From Laboratory Practice to Industrial Applications. Org. Process Res. Dev. 2017, 21 (9), 1213–1226. https://doi.org/10.1021/acs.oprd.7b00004.

(29) Lowe, D. Electrochemistry For All https://blogs.sciencemag.org/pipeline/archives/2017/08/24/electrochemistry-for-all.

(30) IKA. IKA ElectraSyn 2.0 https://www.ika.com/en/Products-Lab-Eq/Electrochemistry-Process-Mass-Intensification.html.

(31) Shono, T.; Matsumura, Y.; Tsubata, K.; Kamada, T.; Kishi, K. Electroorganic Chemistry. 116. Electrochemical Transformation of Aldoximes to Nitriles Using Halogen Ions as Mediators: Intermediary Formation of Nitrile Oxides. J. Org. Chem. 1989, 54 (9), 2249–2251. https://doi.org/10.1021/jo00270u044.

(32) Bégé, J.-P.; Bonnet-Depont, D.; Crouse, B. Fluorinated Alcohols: A New Medium for Selective and Clean Reaction. Synlett 2004, 2004 (1), 18–29. https://doi.org/10.1055/s-2003-44973.

(33) Huisgen, R.; Bihlmaier, W.; Geittner, J.; Reissig, H.-U. The Stereospecificity of Diazomethane Cycloadditions. Heterocycles 1978, 10 (1), 147. https://doi.org/10.3987/S-1978-01-0147.

(34) ACS. Process Mass Intensity Tool https://www.acs.org/tech/acs-process-mass-intensity-tool.

(35) Zhao, G.; Liang, L.; Wen, C. H. E.; Tong, R. In Situ Generation of Nitrile Oxides from NaCl–Oxone Oxidation of Various Aldoximes and Their 1,3-Dipolar Cycloaddition. Org. Lett. 2019, 21 (1), 315–319. https://doi.org/10.1021/acs.orglett.8b03829.

(36) Tools for Innovation in Chemistry https://www.acsorg.com/maximum-precision-devices-for-application/

(37) Anastas, P. T.; Warner, J. C. Green Chemistry: Theory and Practice; Oxford University Press: New York, 1998.

(38) Cardoso, D. S. P.; Sljukić, B.; Santos, D. M. F.; Sequeira, C. A. C. Organic Electrosynthesis: From Laboratory Practice to Industrial Applications. Org. Process Res. Dev. 2017, 21 (9), 1213–1226. https://doi.org/10.1021/acs.oprd.7b00004.

(39) Lowe, D. Electrochemistry For All https://blogs.sciencemag.org/pipeline/archives/2017/08/24/electrochemistry-for-all.

(40) IKA. IKA ElectraSyn 2.0 https://www.ika.com/en/Products-Lab-Eq/Electrochemistry-Process-Mass-Intensification.html.

(41) Shono, T.; Matsumura, Y.; Tsubata, K.; Kamada, T.; Kishi, K. Electroorganic Chemistry. 116. Electrochemical Transformation of Aldoximes to Nitriles Using Halogen Ions as Mediators: Intermediary Formation of Nitrile Oxides. J. Org. Chem. 1989, 54 (9), 2249–2251. https://doi.org/10.1021/jo00270u044.

(42) Bégé, J.-P.; Bonnet-Depont, D.; Crouse, B. Fluorinated Alcohols: A New Medium for Selective and Clean Reaction. Synlett 2004, 2004 (1), 18–29. https://doi.org/10.1055/s-2003-44973.

(43) Huisgen, R.; Bihlmaier, W.; Geittner, J.; Reissig, H.-U. The Stereospecificity of Diazomethane Cycloadditions. Heterocycles 1978, 10 (1), 147. https://doi.org/10.3987/S-1978-01-0147.

(44) ACS. Process Mass Intensity Tool https://www.acs.org/tech/acs-process-mass-intensity-tool.

(45) Zhao, G.; Liang, L.; Wen, C. H. E.; Tong, R. In Situ Generation of Nitrile Oxides from NaCl–Oxone Oxidation of Various Aldoximes and Their 1,3-Dipolar Cycloaddition. Org. Lett. 2019, 21 (1), 315–319. https://doi.org/10.1021/acs.orglett.8b03829.

(46) Bégé, J.-P.; Bonnet-Depont, D.; Crouse, B. Fluorinated Alcohols: A New Medium for Selective and Clean Reaction. Synlett 2004, 2004 (1), 18–29. https://doi.org/10.1055/s-2003-44973.

(47) Huisgen, R.; Bihlmaier, W.; Geittner, J.; Reissig, H.-U. The Stereospecificity of Diazomethane Cycloadditions. Heterocycles 1978, 10 (1), 147. https://doi.org/10.3987/S-1978-01-0147.