Enzymatic C-N Bond Formation via Aerobic Nitroso Ene Reactions

Jan Deska, Christina Jäger

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Enzymatic C-N Bond Formation via Aerobic Nitroso Ene Reactions

Christina Jäger,^a and Jan Deska^{a*}

(a) Department of Chemistry, Aalto University, Espoo, Finland

jan.deska@aalto.fi

Abstract: A novel biocatalytic protocol enables the direct and selective introduction of nitrogen functionalities via activation of allylic C-H bonds. Utilizing an oxidase/peroxidase couple for the formal dehydrogenation of N-hydroxycarbamates and hydroxamic acids with air as terminal oxidant, acylnitroso species are generated under particularly mild aqueous conditions. The reactive intermediates undergo C-N bond formation through an ene-type mechanism and provide high yields both in intramolecular and intermolecular enzymatic aminations. Investigations on alternative reaction pathways and labelling studies provide more insights into this unprecedented biocatalytic promiscuity of classical oxidoreductases as catalysts for nitroso ene-based transformations.

Nature's repertoire to introduce nitrogen moieties into molecular frameworks is generally limited to direct functional group interconversions. In addition to the well-developed amide-forming enzymes, the toolbox of synthetically valuable biocatalysis methods has been expanded steadily in the past years through a number of nitrogen-fixation strategies based on reductive aminations and additions catalyzed by transaminases, imine reductases, and amine dehydrogenases, to name a few.^[1] In a complementary approach mimicking chemistry by biological means, most recently the biocatalytic imitation of non-natural but synthetically relevant reactions was moved into the spotlight,^[2] and in particular nitrene insertion-based transformations utilizing engineered heme proteins introduced whole new pathways for the biocatalytic C-N bond formation.^[3]

As a yet untapped template from the world of traditional synthesis, another attractive alternative approach for selective C-N bond formations engages reactive nitroso species as electrophiles with allylic functionalities. First explored in the 1960's for the synthesis of allylamines using nitrosobenzene,^[4] nitroso ene reactions, and the related nitroso-Diels-Alder cycloadditions, are nowadays found in a wide variety of synthetic strategies towards natural products and pharmaceuticals.^[5] Due to their high reactivity, nitroso reagents are most commonly created in situ through oxidation of amines or hydroxylamines, or the reduction of nitro moieties.^[6] Among the different derivatives, particularly acylnitroso species have been proven as versatile reaction partners, as these electrophiles offer a

a amine synthesis through functional group interconversion (natural mode)

b C-N bond formation via nitrene C-H insertion (reverse biomimetics)

c nitroso ene-type amination by promiscuous oxidoreductases (this work)

Scheme 1. Approaches for C-N bond formation mediated by enzyme catalysts: a) established biocatalytic amination through transferases and oxidoreductases; b) designer biocatalysts enabling nitrene insertion chemistry; c) ene-type functionalization by laccases and peroxidases.

good compromise between reactivity and selectivity.^[7] As the C-N bond formation through nitroso ene reactions proceeds with specificity on allylic fragments even on more functionalized molecules, a variety of different methodologies for the synthesis and trapping of acylnitroso species have been developed over the years. Common approaches include the thermal dissociation,^[8] photooxidations,^[9] or the application stoichiometric oxidizing agents the likes of peroxides,^[10] diacetoxyiodobenzene,^[11] NMO,^[12] and periodates.^[13] Logically, those methods were subsequently supplemented by a variety of catalytic versions employing transition metal complexes as mediators for the oxidative or reductive generation of the nitroso electrophiles, covering protocols based on iridium, ruthenium, rhodium, molybdenum or palladium.^[14] Particularly mild conditions have been achieved by rather simple catalytic systems utilizing copper^[15] or iron^[16] catalysts in combination with oxygen or hydrogen peroxide as terminal oxidants which provide effective C-N bond forming reactions both in intra- and intermolecular reactions. Although singular examples of catalytic-enantioselective methods have been reported for nitroso-Diels-Alder cycloadditions,^[17] the application of the nitroso ene reaction for the synthesis of optically active

amines has so far been limited to auxiliary-controlled diastereoselective transformations.^[15c,18] In our endeavor to discover new enzymatic tools that are inspired by traditional organic chemistry, we envisaged that oxidative biocatalysts could act as mediators for this attractive C-N-coupling reaction. While ensuring mild conditions and benign oxidants, the potential enzyme catalysts would need to provide a protective environment for the reactive nitroso electrophiles, that, if successful, could also serve as starting point for protein engineering efforts to develop enantioselective biocatalyst variants.

With both copper and iron-based homogeneous systems described in literature, our investigation on the biocatalytic nitroso ene reaction started with a broad screening over a series of redox-active metalloproteins featuring either of these two elements as active site motif. As one of the most prominent copper-dependent enzyme classes with precedence as tool in organic synthesis, oxygen-activating laccases were initially taken into the focus of our study. While laccase from *Rhus vernicifera* did not engage in any kind of activation of the model substrate **1a** (Table 1, entry 1), the multi-copper proteins from *Agaricus bisporus*, *Myceliophthora thermophila*, *Trametes versicolor* and *Pleurotus ostreatus* could all in fact transform the *N*-hydroxy carbamate and provide **2a** in mediocre to acceptable yields (Table 1, entries 2-5). The contrast between full conversion and low yields however raises the question of selectivity and it appears that subsequent further oxidation and decomposition of the *N*-hydroxy oxazolidinone **2a** may hamper the process. The well-established laccase from *T. versicolor* distinguished itself as most effective copper protein, but variability in isolated yields of **2a** raised the question of reliability of a black box catalyst (Table 1, entry 5). Studies on the true role of laccases in this transformation are currently part of a more biotechnological investigation that is however outside the scope of this article. Tyrosinase as alternative oxidizing copper protein proved to be inactive in the desired reaction (Table 1, entry 6). Succeeding those somewhat ambiguous results, we turned our attention to a bi-enzymatic system that was previously employed by us successfully in the aerobic oxidative transformation of furans and allenes.^[19] Combining glucose oxidase (GOx) as oxygen-activating biocatalyst with peroxidases or peroxygenases to mediate the oxidation of the substrate, we imagined that also these biocatalysts could generally qualify to *in situ* generate the reactive acylnitroso species. In presence of glucose, GOx and air, little to no product formation was observed with peroxygenase and lactoperoxidase (Table 1, entries 7 & 8). In contrast, with chloroperoxidase from *C. fumago* full conversion of **1a** was achieved within 29 hours. A yield of 64% of **2a** however indicated that also with this biocatalyst, similar to the most effective laccases, overoxidation remained a problem (Table 1, entry 9). Gratifyingly, supplementing horseradish peroxidase (HRP) resulted in not only fast conversion but also an excellent isolated yield of 97% (Table 1, entry 10). Although solubility of **1a** in the phosphate buffer was not an issue, cosolvent solutions were taken into consideration, as structural variations on related

N-hydroxy carbamates and hydroxamic acids could affect substrate lipophilicity unfavorably. The GOx/HRP system proved to be very robust and neither addition of the water-miscible dioxane nor the two-phase solution featuring ethyl acetate caused significant deterioration of reactivity or selectivity (Table 1, entry 11 & 12). In absence of added peroxidase, no reaction was taking place, ruling out any action by the glucose oxidase additive itself (Table 1, entry 13).

Table 1. Enzyme-mediated oxidation-induced cyclization of **1a**.

#	enzyme	additive	cosolvent ^[a]	time [h]	yield [%] ^[b,c]
1	laccase (<i>R. vernicifera</i>)	–	–	48	0 (0)
2	laccase (<i>A. bisporus</i>)	–	–	23	13 (100)
3	laccase (<i>M. thermophila</i>)	–	–	6	28 (100)
4	laccase (<i>P. ostreatus</i>)	–	–	5	61 (100)
5	laccase (<i>T. versicolor</i>)	–	–	6	63 (100)
6	tyrosinase (<i>A. bisporus</i>)	–	–	48	0 (0)
7	peroxygenase (<i>A. aegerita</i>)	D-Glc, GOx (<i>A. niger</i>)	–	48	0 (0)
8	lactoperoxidase (bovine)	D-Glc, GOx (<i>A. niger</i>)	–	72	<5 (50)
9	chloroperoxidase (<i>C. fumago</i>)	D-Glc, GOx (<i>A. niger</i>)	–	29	64 (100)
10	peroxidase (from horseradish)	D-Glc, GOx (<i>A. niger</i>)	–	2	97 (100)
11	peroxidase (from horseradish)	D-Glc, GOx (<i>A. niger</i>)	EtOAc	2	96 (100)
12	peroxidase (from horseradish)	D-Glc, GOx (<i>A. niger</i>)	dioxane	2	94 (100)
13	none	D-Glc, GOx (<i>A. niger</i>)	–	48	0

Reaction conditions, for laccases/tyrosinase: **1a** (10 mM), laccase or tyrosinase (100 U), phosphate buffer (pH 7.0, 100 mM, 7 mL), 25 °C; for peroxygenases: **1a** (10 mM), D-glucose (50 mM), glucose oxidase (70 U), peroxidase (70 U), phosphate buffer (pH 7.0, 100 mM, 7 mL), 25 °C. [a] 10 vol%. [b] Isolated yields of **2a**. [c] Conversion in parenthesis.

Apparently, the conversion of **1a** to **2a** includes formation of an asymmetric centre and naturally, enantioselectivity is almost taken for granted in biocatalysis. A strong stereochemical induction, however,

would require a distinct Lewis-acidic binding site for the reactive nitroso intermediate, that, given the nature of activation by peroxidases through electron transfer into compound **1**, is unavailable in the heme proteins. In contrast, the laccase's mononuclear T1 copper may indeed act as substrate binding motif. Consequently, all peroxidase-catalyzed reactions yielded racemic products whereas moderate enantiomeric excesses from up to 72% were achieved with laccase from *T. versicolor*. Thus, an enantioselective methodology for the nitroso ene reaction will likely rely on future protein engineering efforts with the blue-copper enzymes.

The peroxidase-based cyclization protocol was subsequently examined regarding the tolerated substrate scope and structural limitations of the methodology (Scheme 2). Changing from the *N*-hydroxy carbamate motif to a hydroxamic acid in **1b**, good reactivity could be maintained and the *N*-hydroxy lactame **2b** was obtained in 77% yield. Similarly, variation of the substitution pattern around the olefinic double bond was generally tolerated as indicated by moderate to very high yields of products **2c-2e**. All reactions proceeded well at room temperature which poses a stark contrast to the original chemical template that required up to 100 °C to achieve comparable yields.^[16] Utilizing chiral *N*-hydroxy carbamates, good to excellent induced diastereoselectivity could be observed and the spirocyclic product **2g** was isolated in 97% yield as 92:8 mixture of diastereomers. In contrast, no reaction was observed for the verbenol-derived **1h** or the 3-methylcinnamyl substrate *E*-**1i**, which could be either attributed to an excessive steric demand or unfavorable geometrical constraints.

Scheme 2. Scope of the biocatalytic aerobic nitroso ene cyclization.

The *cis* or *trans* arrangement between the intermediate nitroso group and the reactive C-H bond across the olefinic double bond has not been recognized as major selectivity factor in previous studies, however, significantly higher reaction temperatures were commonly applied in classical, chemical nitroso ene reactions. To get a clearer picture on the potential geometrical bias of the HRP-catalyzed cyclization, the two isomeric 2-hexenyl *N*-hydroxy carbamates *E*-**1j** and *Z*-**1j** were reacted under identical conditions (Scheme 3). Here, the *E*-configured isomer delivered the expected oxazolidinone **2j** in a good yield of 66%. With a rotationally flexible CH₂ unit in allylic position, the product was obtained as a 3-to-1 *E/Z*-mixture. Quite differently, no cyclization product could be detected with the opposite stereoisomeric substrate and *Z*-**1j** was instead quantitatively decomposed.

Scheme 3. Selectivity in the biocatalytic conversion of olefinic isomers.

The half-life of acylnitroso species in the aqueous reaction medium is likely limited as they are generally characterized as short-lived electrophiles. In a recent study by Goldstein,^[20] the rates of hydrolysis of *in situ* prepared nitroso carbonyls, and reactions with other nucleophiles, were investigated and half-lives of a few minutes were observed. In the HRP-catalyzed cyclization, the intramolecular nature and a potential protective function of the protein may explain the good to excellent yields. When moving to an intermolecular system, however, the exposure of the reactive intermediate is unavoidable. Not surprisingly, initial attempts to conduct a bimolecular coupling between *N*-hydroxy carbamate **3** and tetramethylethylene (**4**) in equimolar amounts under the previously optimized conditions with the oxidase/ peroxidase couple resulted in full conversion in 2 h, but with a mediocre yield of only 30%. To our delight, simply swapping the organic cosolvent from ethyl acetate to tetramethylethylene itself resulted in identical kinetics and disappearance of **3** within 2 h. In this case however, the C-N-coupling product **5** was isolated in an excellent yield of 86% (Scheme 4). Though practical for some olefinic reaction partners, the use of excess reactants is certainly not the most elegant approach.

Scheme 4. Enzymatic intermolecular nitroso ene reaction.

Thus, it will be necessary to further adjust the reaction conditions for this intermolecular nitroso ene reaction, either through reaction medium engineering,^[19c] or through modulation of the reactivity by variation of the electronics of the enophile.^[7]

With a highly effective biocatalytic protocol in hand to prepare and react acylnitroso species *in situ* under mild aerobic conditions, the extension to related reactions as probe for the synthetic potential of this enzymatic tool appeared to be a logical next step. With a proven activity in both intra- and intermolecular ene-type reaction, we assumed that the peroxidase could likewise act as catalyst in nitroso-Diels-Alder reactions. Utilizing *N*-hydroxycarbamate **1n** featuring a diene moiety and a geometry that would rule out competing ene reactivities, our established method indeed translated well into an intramolecular [4+2] cycloaddition yielding bicycle **5** as major product (Scheme 5a).

More surprisingly, the peroxidase-mediated oxidation of the benzoate-derived hydroxamic acid **1l** resulted in a slightly different reaction outcome, providing amidohydroxylated species **7** selectively as the sole product, alongside with unreacted starting material (Scheme 5b). Even though similar *N,O*-difunctionalizations of olefins are not unknown,^[21] to the best of our knowledge, this bifurcation has so far not been observed in the previous nitroso ene studies and could indicate an interesting additional avenue for future investigations. Noteworthy, none of the other substrates tested in this work have led to similar amidohydroxylation products. While the current yield of 20% would certainly require more optimization efforts to present the reaction as serious synthetic method, this deviation from the expected product outcome might help to shed some light on potential pathways of the nitroso ene reaction under the herein described aqueous enzymatic conditions (*vide infra*).

Scheme 5. Cycloaddition and amidohydroxylation-selective conversions through HRP/GOx-mediated aerobic oxidation.

While the nitroso-Diels-Alder and the nitroso-ene reactions are identical in how the reactive R-NO species are formed, the ene-type reaction is believed to follow a stepwise reaction pathway as opposed to the concerted

[4+2] cycloaddition of the Diels-Alder mechanism. The underlying process of these kinds of ene reactions is still under debate, and several feasible mechanisms have been proposed over the years. Most plausible pathways, based on experimental and/or computational studies, are proceeding via zwitterionic or diradical intermediates, concerted pathways, as well as via intermediary aziridine *N*-oxides (ANO).^[22-26] Although not unchallenged, the current established view assumes a combination of hypotheses with a polarized diradical pathway that does not rule out the ANO as an ‘innocent bystander’.^[23,26] However, these mechanistic studies mostly refer to the intermolecular ene reaction of nitroso compounds, and are likely not fully transferable to our system due to the unusual aqueous reaction environment.

In order to gain some insights into mechanistic details of the uncommon aqueous nitroso ene chemistry, a series of isotopically labelled substrates were prepared to study the intramolecular conversion of **1a** to the cyclic *N*-hydroxycarbamate **2a**. A symmetrically deuterated derivative **1a-d₆** was investigated, and comparison of initial rates of conversion of deuterated and non-deuterated substrates revealed a strong inverse isotope effect ($k_H/k_D = 0.79$) (Scheme 2a). This isotope effect likely arises from the hybridization change of carbon C2 in the double bond (sp^2 to sp^3) during the rate-limiting step. Similar inverse secondary effects have not been previously observed experimentally in nitroso ene reaction, though they were also not ruled out in the first place. As competing diradical or dipolar pathways would most certainly lead to more significant normal isotope effects, it is believed that a β secondary isotope effect could not be recognized.^[22-26] These expected effects of diradical or non-ANO zwitterionic pathways are therefore not consistent with our observation leading to the exclusion of these mechanistic options. Hence, the formation of an ANO as an intermediate instead of an inoperative bystander could be the logic conclusion. The absence of any primary KIE indicates a stepwise mechanism with irreversible formation of an intermediate in the rate-limiting step followed by the hydrogen migration. Similar effects were observed by Orfanopoulos in the ene reaction of triazolidinediones with phorone-*d*₆ and styrene-like substrates, who concluded an S_N2 -type addition of the N=N enophile to the double bond forming a three-membered ANO-like intermediate in the rate-determining step.^[27]

The clear on/off-selectivity in the conversion of stereochemically defined isomers (*E* vs *Z*) was already illustrated in Scheme 3; a feature that is in line with a geometrically rigid intermediate such as the aziridine *N*-oxide. At the same time, this specificity stands in contrast to other protocols that do allow the transformation of *Z*-olefins in intramolecular nitroso ene reactions, supporting the possible deviation in pathways between polar-protic and the more commonly used lipophilic media. To probe the expected selectivity for the cleavage of *E*-oriented C-H bonds also in a sterically truly unbiased system, we furthermore synthesized the unsymmetrically labelled *Z*-**1a-d₃** and subjected it to the established GOx/HRP-

catalyzed cyclization. The thus obtained oxazolidinone **2a-d₃** carried the deuterium label specifically in the remaining methyl group (Scheme 6b). Likewise, specific formation of the methylene-labelled **2a-d₂** was observed when starting from the isomeric *E*-**1a-d₃**.

Scheme 6. The HRP-mediated cyclization of isotopomers of **1a** provides hints on the underlying reaction pathway of aqueous nitroso ene reactions.

Combining the different findings – the inverse KIE, the stereospecificity and the unexpected formation of amidohydroxylation product **7** – the previously proposed aziridine *N*-oxide as relevant reaction intermediate seems plausible providing a rational for both the unusual limitation to *E*-configured olefins and the bifurcation via water addition to the electrophilic aziridinium species (Scheme 6c).

To conclude, a very mild *C-N* bond-forming method based on a nitroso ene pathway was developed, utilizing a robust and reliable enzymatic couple consisting of horseradish peroxidase and glucose oxidase. As another important example for catalytic promiscuity, the biocatalytic protocol generates and transforms reactive acylnitroso intermediates in a highly selective manner, allowing both intra- and intermolecular aminations to take place with high yields in an aqueous medium. Our study further revealed that also certain laccases provide the

necessary redox profile and protective environment to conduct nitroso ene-type transformations and offers a first blueprint for the engineering of enantioselective versions of this reaction. In addition to the promising starting point for stereochemical optimizations, the observation of side reactivities such as [4+2] cycloadditions and oxidative 1,2-amidohydroxylations underline the yet untapped potential of this unprecedented biocatalytic activation pathway that will be addressed in much more detail in our future investigations.

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Keywords

biocatalysis • ene reaction • heterocycles • amination • cyclization

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Electronic Supporting Information

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Table of Contents

1. General Remarks.....	3
2. Procedures and analytical data.....	4
2.1 Synthesis of hydroxamic acids.....	4
2.2 Enzymatic intramolecular nitroso ene reactions using GOx/HRP.....	14
2.3 Enzymatic intramolecular nitroso-Diels-Alder reactions using GOx/HRP.....	19
2.4 Enzymatic intermolecular nitroso ene reactions using GOx/HRP.....	19
3. Peroxidase Screening.....	20
4. Laccase Screening.....	21
5. Kinetic isotope studies.....	22
6. NMR spectra.....	23
7. Supplementary references.....	51

1. General Remarks

Commercially available reagents were used without further purification. Acetone-*d*6 was purchased from eurisotop. Glucose oxidase (type II, *Aspergillus niger*), horseradish peroxidase, chloroperoxidase (*Caldariomyces fumago*), bovine lactoperoxidase and all laccases were purchased from Sigma Aldrich.

All reactions were carried out under argon atmosphere if not stated different and performed with dry solvents. Solvents were dried with the help of a solvent drying system MB-SPS-800 from M. Braun. All enzymatic reactions were carried out under non-inert conditions.

Silica gel from Merck (Millipore 60, 40-60 μm , 240-400 mesh) was used for column chromatography and silica pad filtrations. Reactions were monitored via thin layer chromatography (TLC) using precoated silica gel plates from Machery-Nagel (TLC Silica gel 60 F₂₅₄). The spots were identified using irradiation with UV light and a staining solution (basic potassium permanganate solution).

¹H- and ¹³C-NMR spectra were measured with a Bruker Avance NEO 400 at 20 °C. The chemical shifts are reported in ppm related to the signal of residual solvent of CDCl₃ (¹H: (CDCl₃) = 7.26 ppm, ¹³C: (CDCl₃) = 77.2 ppm). Infrared spectra were recorded on a Bruker Alpha Eco ATR FTIR device. High resolution mass spectrometry was performed on an Agilent 6530 QTOF spectrometer. Melting points were measured on a Bibby Scientific Stuart melting point SMP30 with an error of ± 0.5 °C. Melting points were determined by three different measurements for one substance obtaining the temperature of the melting start and the fully melted state. The median of these two temperatures is considered to be the melting point. The average of the three measured melting points is the one reported in this supporting information.

2. Procedures and Analytical Data

2.1 Synthesis of hydroxamic acids

Representative general procedure: To a 0.2 M solution of alcohol (1.0 eq.) in dry acetonitrile was added carbonyl diimidazole (CDI) (1.5 eq.) under argon atmosphere. After full conversion of the alcohol (followed by TLC) imidazole (4.0 eq) and hydroxylamine hydrochloride (5.0 eq.) were added. After observing full conversion of the intermediate, the solvent was removed under reduced pressure. The resulted solid was dissolved in EtOAc and 1M HCl solution. The phases were separated and the aqueous phase was washed 3x with EtOAc. The combined organic phases were dried over Na₂SO₄, filtered and the solvent was removed under reduced pressure. The crude was purified via flash column chromatography on silica gel.^[1]

3-methylbut-2-en-1-yl *N*-hydroxycarbamate (**1a**)

According to the representative general procedure, 3-methylbut-2-en-1-ol (1.2 mL, 1.03 g, 11.98 mmol) was reacted. The crude was purified via column chromatography on silica gel (4:1 *n*-Hep/EtOAc; *R*_f = 0.32 (3:2 *n*-Hep/EtOAc)) to yield **1a** as a colorless oil (1.62 g, 11.18 mmol, 145.16 g/mol, 93 %). Spectral data are in agreement with literature precedent.^[1]

¹H-NMR (400 MHz, CDCl₃) δ [ppm] = 7.23 (s, 1H, -NH-), 6.85 (bs, 1H, -OH), 5.41-5.27 (m, 1H), 4.65 (d, *J* = 7.3 Hz, 2H), 1.76 (d, *J* = 0.8 Hz, 3H), 1.71 (d, *J* = 0.9 Hz, 3H). **¹³C-NMR** (100 MHz, CDCl₃) δ [ppm] = 159.5, 140.2, 118.1, 63.1, 25.8, 18.1. **FT-IR** (ATR) ν [cm⁻¹] = 3290 (w), 2933 (w), 1706 (s), 1445 (m), 1259 (m), 1101 (s).

3-(methyl-d₃)-4,4,4-d₃-but-2-en-1-yl *N*-hydroxycarbamate (**1a-d₆**)

Step 1/3: To a solution of triethyl phosphonoacetate (4.1 mL, 20.4 mmol, 1.5 eq.) in 35 mL THF was added *n*-BuLi (7.6 mL, 1.4 eq, 2.5 M in hexanes) at 0 °C. After full addition, the reaction mixture was allowed to warm to room temperature. After 30 min acetone-*d*₆ (1 mL, 13.6 mmol, 1.0 eq.) was added. After 5 h, the reaction was quenched with saturated NH₄Cl solution. The aqueous phase was extracted 3x with Et₂O. The combined organic phases were washed with brine, dried over Na₂SO₄, filtered and the solvent was removed under reduced pressure. The crude was purified via column chromatography on silica gel (2:1 *n*-Pen/Et₂O) to yield ethyl 3-(methyl-d₃)- 4,4,4-d₃-but-2-enoate (1.54 g, 11.5 mmol, 134.21 g/mol, 85 %) Spectral data are in agreement with literature precedent.^[8]

¹H-NMR (400 MHz, CDCl₃): δ [ppm] = 5.67 (s, 1H), 4.14 (q, *J* = 7.1 Hz, 2H), 1.27 (t, *J* = 7.1 Hz, 3H).

Step 2/3: To a solution of 3-(methyl-d₃)-4,4,4-d₃-but-2-enoate (1.12 g, 8.9 mmol, 1.0 eq.) in 10 mL DCM was added DIBAL-H (27 mL, 3.0 eq., 1 M in *c*-Hex) at 0 °C. After 2 h the reaction was quenched with

MeOH. The mixture was poured to 30 mL Et₂O and 30 mL of 2 M HCl solution was added. The aqueous phase was extracted 3x with Et₂O. The combined organic layers were washed with 1 M HCl solution and 2x with brine, dried over Na₂SO₄, filtered and the solvent was removed under reduced pressure. The crude was purified via bulb-to-bulb distillation to afford 3-(methyl-d₃)-4,4,4-d₃-but-2-en-1-ol (450 mg, 4.9 mmol, 92.17 g/mol, 55 %). Spectral data are in agreement with literature precedent.^[9]

¹H-NMR (400 MHz, CDCl₃): δ [ppm] = 5.41 (t, *J* = 7.1 Hz, 1H), 4.12 (d, *J* = 7.0 Hz, 2H), 1.21 (s, 1H, -OH).

Step 3/3: According to the representative general procedure, 3-(methyl-d₃)-4,4,4-d₃-but-2-en-1-ol (200 mg, 2.2 mmol) was reacted. The crude was purified via column chromatography on silica gel (2:1 *n*-Pen/Et₂O; *R*_f = 0.15 (2:1 *n*-Hep/EtOAc)) to yield **1a-d₆** as a colorless oil (151.2 mg, 1.5 mmol, 229.4 g/mol, 69 %, D₆ ≥ 99 %).

¹H-NMR (400 MHz, CDCl₃): δ [ppm] = 7.26 (s, 1H, -NH-), 7.06 (s, 1H, -OH), 5.34 (t, *J* = 7.3 Hz, 1H), 4.65 (d, *J* = 7.3 Hz, 2H). **FT-IR** (ATR) ν [cm⁻¹] = 3294 (w), 2958 (vw), 1705 (m), 1463 (w), 1257 (s), 1104 (s). **HRMS** (ESI): *m/z* [M+Na⁺] calcd for C₆H₅D₆NO₃: 174.1008; found: 174.1014.

***E*-3-methyl-4,4,4-d₃-but-2-en-1-yl *N*-hydroxycarbamate (*E*-1a-d₃)**

Step 1/4: To a solution of thiophenol (1.7 g, 15.44 mmol, 1.2 eq.) in 30 mL EtOH was added NaOH (618 mg, 15.44 mmol, 1.2 eq.). After stirring for 30 min at room temperature, ethyl but-2-ynoate (1.5 mL, 12.87 mmol, 1.0 eq.) in 5 mL EtOH was added. After 4 h the reaction was slightly acidified with diluted acetic acid. The aqueous phase was extracted 3x with Et₂O and the combined organic phases were washed with 4 % NaOH solution, H₂O, brine and dried over Na₂SO₄, filtered and the solvent was removed under reduced pressure. The crude was purified via column chromatography (*n*-Hep/EtOAc 30:1; *R*_f = 0.54 & 0.44 (6:1 *n*-Hep/EtOAc)) to yield and separate ethyl *Z*-3-(phenylthio)but-2-enoate (460 mg, 2.07 mmol, 222.3 g/mol 16 %) and ethyl *E*-3-(phenylthio)but-2-enoate (1.88 g, 8.47 mmol, 222.3 g/mol 66 %). Spectral data are in agreement with literature precedent.^[18]

E-Isomer: **¹H-NMR** (400 MHz, CDCl₃): δ [ppm] = 7.45-7.41 (m, 2H), 7.39-7.34 (m, 3H), 5.19 (d, *J* = 1.1 Hz, 1H), 4.02 (q, *J* = 7.1 Hz, 2H), 2.37 (d, *J* = 1.1 Hz, 3H), 1.15 (t, *J* = 7.1 Hz, 3H). **¹³C-NMR** (100 MHz, CDCl₃): δ [ppm] = 165.6, 159.8, 135.6, 130.0, 129.9, 111.1, 59.7, 20.2, 14.4.

Z-Isomer: **¹H-NMR** (400 MHz, CDCl₃): δ [ppm] = 7.57-7.48 (m, 2H, -Ph), 7.45-7.32 (m, 3H, -Ph), 5.85 (d, *J* = 1.1 Hz, 1H, H-2), 4.22 (q, *J* = 7.1 Hz, 2H, H-5), 1.81 (d, *J* = 1.1 Hz, 3H, H-4), 1.30 (t, *J* = 7.1 Hz, 3H, H-6). **¹³C-NMR** (100 MHz, CDCl₃): δ [ppm] = 166.3, 158.3, 136.1, 130.9, 129.5, 129.1, 111.9, 59.9, 25.1, 14.4.

Step 2/4: CuI (648 mg, 3.4 mmol, 2.0 eq.) was dispensed in 18 mL THF. CD₃MgI (9 mL, 5.0 eq., 1M in Et₂O) was slowly added at -78 °C and the reaction mixture was stirred for 15 min at that temperature. Ethyl *E*-3-(phenylthio)but-2-enoate (400 mg, 1.8 mmol, 1.0 eq.) in 9 mL THF was slowly added at -78 °C. The reaction mixture was stirred for 8 h at the same temperature and was then quenched with saturated NH₄Cl solution at 0 °C. The aqueous phase was extracted 2x with Et₂O. The combined organic phases

were washed with 5 % NaOH solution, H₂O, brine and dried over Na₂SO₄, filtered and the solvent was removed under reduced pressure (max. 350 mbar at 30 °C). The crude was purified via column chromatography (*n*-Pen/Et₂O 30:1; R_f = 0.76 (2:1 *n*-Hep/EtOAc)) to yield ethyl *E*-3-methyl-4,4,4-d₃-but-2-enoate in residual Et₂O. Spectral data are in agreement with literature precedent.^[18]

¹H-NMR (400 MHz, CDCl₃): δ [ppm] = 5.66 (d, *J* = 1.0 Hz, 1H), 4.13 (q, *J* = 7.1 Hz, 2H), 2.15 (d, *J* = 1.2 Hz, 3H), 1.26 (t, *J* = 7.1 Hz, 3H).

Step 3/4: To a solution of the obtained ethyl *E*-3-methyl-4,4,4-d₃-but-2-enoate in residual Et₂O and 1 mL DCM was added DIBAL-H (3.2 mL, 3.0 eq., 1M in *c*-Hex) at 0 °C. After full conversion (followed by TLC), the reaction was quenched with MeOH followed by 1M HCl. The aqueous phase was extracted 2x with Et₂O. The combined organic phases were washed with brine, dried over Na₂SO₄, filtered and the solvent was removed under reduced pressure (volatile!). The crude was purified via bulb to bulb distillation to yield *E*-3-methylbut-2-en-4,4,4-d₃-1-ol in residual Et₂O. Spectral data are in agreement with literature precedent.^[18]

¹H-NMR (400 MHz, CDCl₃): δ [ppm] = 5.41 (td, *J* = 7.1, 1.3 Hz, 1H), 4.13 (d, *J* = 7.1 Hz, 2H), 1.68 (s, 3H).

Step 4/4: According to the representative general procedure, *E*-3-methylbut-2-en-4,4,4-d₃-1-ol in residual Et₂O was reacted. The crude was purified via column chromatography on silica gel (3:1 *n*-Hep/EtOAc; R_f = 0.21 (2:1 *n*-Hep/EtOAc)) to yield *E*-**1a-d₃** as a colorless oil (49 mg, 10.33 mmol, 148.18 g/mol, 18 % over 3 steps, D₃ ≥ 95 %).

¹H-NMR (400 MHz, CDCl₃): δ [ppm] = 7.18 (s, 1H), 6.58 (s, 1H), 5.35 (td, *J* = 7.3, 1.2 Hz, 1H), 4.66 (d, *J* = 7.3 Hz, 2H), 1.72 (d, *J* = 0.7 Hz, 3H). **FT-IR** (ATR) ν [cm⁻¹] = 3295 (w), 2917 (vw), 1705 (m), 1456 (w), 1257 (m), 1102 (s). **HRMS** (ESI): *m/z* [M+H⁺] calcd for C₆H₈D₃NO₃: 149.1000; found: 149.1007.

Z-3-methyl-4,4,4-d₃-but-2-en-1-yl *N*-hydroxycarbamate (Z-1a-d₃**)**

Z-3-methylbut-2-en-4,4,4-d₃-1-ol was synthesized from ethyl *Z*-3-(phenylthio)but-2-enoate like described before.^[18] According to the representative general procedure, *Z*-3-methylbut-2-en-4,4,4-d₃-1-ol in residual Et₂O was reacted. The crude was purified via column chromatography on silica gel (3:1 *n*-Hep/EtOAc; R_f = 0.37 (1:1 *n*-Hep/EtOAc)) to yield **1a-Z-d₃** as a colorless oil (75 mg, 0.5 mmol, 148.18 g/mol, 28 % over 3 steps, D₃ ≥ 95 %).

¹H-NMR (400 MHz, CDCl₃): δ [ppm] = 7.21 (s, 1H), 6.73 (s, 1H), 5.35 (t, *J* = 7.3 Hz, 1H), 4.65 (dd, *J* = 7.3, 0.7 Hz, 2H), 1.76 (s, 3H). **FT-IR** (ATR) ν [cm⁻¹] = 3298 (w), 2915 (vw), 1705 (m), 1445 (w), 1289 (m), 1112 (s). **HRMS** (ESI): *m/z* [M+H⁺] calcd for C₆H₈D₃NO₃: 149.1000; found: 149.0986.

***N*-hydroxy-5-methylhex-4-enamide (1b)**

Methyl 5-methylhex-4-enoate was prepared from 2-methylbut-3-en-2-ol according to literature precedent.^[17] To 30 mL of a 2 M solution of hydroxylamine hydrochloride in MeOH was added 30 mL of a 4 M solution of KOH in MeOH at 0 °C. The formed KCl was filtered off and the mixture was added to methyl 5-methylhex-4-enoate (1.0 g, 7.03 mmol) and adjusted to pH 10.0 using a solution of KOH in MeOH. The reaction mixture was stirred for 4 h and then adjusted to pH 5.0 with a 5 M HCl solution. The solvent was removed under reduced pressure and the resulting solid was dissolved in MeOH. Remaining insoluble solid was removed via filtration. The solvent was again removed under reduced pressure. This step was repeated with DCM. The crude was purified via column chromatography (DCM + 4 % MeOH; R_f = 0.28 (DCM + 3 % MeOH)) to yield **1b** as a white solid (338.3 mg, 2.36 mmol, 143.19 g/mol, 34 %). Spectral data are in agreement with literature precedent.^[4]

¹H-NMR (400 MHz, CDCl₃): δ [ppm] = 8.54 (bs, 2H, -NH-, -OH), 5.06 (t, J = 6.7 Hz, 1H), 2.32 (q, J = 7.0 Hz, 2H), 2.18 (t, J = 7.2 Hz, 2H), 1.69 (s, 3H), 1.61 (s, 3H). **¹³C-NMR** (100 MHz, CDCl₃): δ [ppm] = 171.3, 134.1, 121.9, 33.2, 25.7, 23.8, 17.7. **FT-IR** (ATR) ν [cm⁻¹] = 3200 (m), 2910 (m), 1624 (vs), 1539 (s), 1067 (s). **Melting point**: 59.8 °C.

***E*-but-2-en-1-yl *N*-hydroxycarbamate (1c)**

According to the representative general procedure, crotyl alcohol (0.5 ml, 5.9 mmol) was reacted. The crude was purified via column chromatography on silica gel (*n*-Hep/EtOAc 4:1; R_f = 0.26 (2:1 *n*-Hep/EtOAc)) to yield **1c** as a colorless oil (49.8 mg, 0.37 mmol, 131.13 g/mol, 6 %). Spectral data are in agreement with literature precedent.^[2]

¹H-NMR (400 MHz, CDCl₃): δ [ppm] = 7.23 (s, 1H, -NH-), 6.72 (bs, 1H, -OH), 5.90-5.76 (m, 1H), 5.65-5.51 (m, 1H), 4.58 (d, J = 6.6 Hz, 2H), 1.72 (dd, J = 6.6, 1.3 Hz, 3H). **¹³C-NMR** (100 MHz, CDCl₃): δ [ppm] = 132.4, 124.6, 66.9, 17.8. **FT-IR** (ATR) ν [cm⁻¹] = 3290 (w), 2945 (vw), 1705 (s), 1449 (m), 1260 (m), 1105 (s).

***E*-2-methylbut-2-en-1-yl *N*-hydroxycarbamate (1d)**

Step 1/2: To a solution of *E*-2-methylbut-2-enal (0.5 mL, 5.17 mmol) in 8 mL MeOH was added NaBH₄ (391 mg, 10.34 mmol) at 0 °C. After 1 h, the reaction mixture was quenched with water and extracted 2x with DCM. The combined organic phases were washed with brine, dried over MgSO₄, filtered and the

solvent was removed under reduced pressure. The crude was purified via bulb-to-bulb distillation to afford *E*-2-methylbut-2-en-1-ol (358 mg, 4.16 mmol, 80 %).

¹H-NMR (400 MHz, CDCl₃): δ [ppm] = 5.57- 5.40 (m, 1H), 3.99 (d, *J* = 3.7 Hz, 2H), 1.67 (s, 3H), 1.62 (ddd, *J* = 6.7, 2.1, 1.0 Hz, 3H), 1.35 (bs, 1H, -OH). **¹³C-NMR** (100 MHz, CDCl₃): δ [ppm] = 135.6, 120.8, 69.2, 13.5, 13.2.

Step 2/2: According to the representative general procedure, *E*-2-methylbut-2-en-1-ol (200 mg, 2.3 mmol) was reacted. The crude was purified via column chromatography on silica gel (*n*-Hep/EtOAc 4:1; *R*_f = 0.21 (2:1 *n*-Hep/EtOAc)) to yield **1d** as a colorless oil (70.3mg g, 0.48 mmol, 145.16 g/mol, 21 %).

¹H-NMR (400 MHz, CDCl₃): δ [ppm] = 7.31 (s, 1H, -NH-), 7.09 (bs, 1H, -OH), 5.67-5.47 (m, 1H), 4.53 (s, 2H), 1.64-1.62 (m, 6H). **¹³C-NMR** (100 MHz, CDCl₃): δ [ppm] = 159.5, 130.4, 125.0, 72.1, 13.5, 13.3. **FT-IR** (ATR) ν [cm⁻¹] = 3289 (w), 2920 (vw), 1704 (s), 1447 (m), 1263 (s), 1100 (s). **HRMS** (ESI): *m/z* [M+Na⁺] calcd for C₆H₁₁NO₃: 168.0631; found: 168.0634.

E-pent-3-en-2-yl *N*-hydroxycarbamate (**1e**)

According to the representative general procedure, *E*-pent-3-en-2-ol (357 mg, 4.2 mmol) was reacted. The crude was purified via column chromatography on silica gel (*n*-Hep/EtOAc 4:1; *R*_f = 0.16 (2:1 *n*-Hep/EtOAc)) to yield **1e** as a white solid (382 mg g, 2.6 mmol, 145.16 g/mol, 63 %). Spectral data are in agreement with literature precedent.^[5]

¹H-NMR (400 MHz, CDCl₃): δ [ppm] = 7.11 (s, 1H, -NH-), 6.43 (s, 1H, -OH), 5.83-5.68 (m, 1H), 5.47 (dddd, *J* = 15.3, 7.0, 3.2, 1.5 Hz, 1H), 5.29 (p, *J* = 6.6 Hz, 1H), 1.69 (d, *J* = 6.5 Hz, 3H), 1.32 (d, *J* = 6.4 Hz, 3H). **¹³C-NMR** (100 MHz, CDCl₃): δ [ppm] = 159.1, 130.2, 129.1, 73.7, 20.4, 17.7. **FT-IR** (ATR) ν [cm⁻¹] = 3343 (w), 3242 (w), 2982 (vw), 1697 (s), 1505 (m), 1276 (m), 1114 (s). **Melting point:** 41.2 °C.

2-cyclohexylideneethyl *N*-hydroxycarbamate (**1f**)

Step 1/3: To a solution of cyclohexanone (3.24 g, 33.0 mmol, 1.0 eq.) in 45 mL toluene was added Methyl (triphenylphosphoranylidene)acetate (13.24 g, 39.6 mmol, 1.2 eq.). The reaction mixture was stirred for 23 h at 110 °C. The solid was filtered off and washed with EtOAc. The solvent was removed under reduced pressure and the resulted solid was again filtered off and washed with EtOAc. The solvent was again removed under reduced pressure and the crude was purified via column chromatography (8:1 *n*-Hep/EtOAc + 3 % TEA) to afford methyl 2-cyclohexylideneacetate as a yellow oil (2.15 g, 14.0 mmol, 42 %). Spectral data are in agreement with literature precedent.^[6]

¹H-NMR (400 MHz, CDCl₃): δ [ppm] = 5.67-5.53 (m, 1H), 3.67 (s, 2H), 2.82 (m, 2H), 2.23-2.14 (m, 2H), 1.70-1.54 (m, 6H). **¹³C-NMR** (100 MHz, CDCl₃): δ [ppm] = 167.4, 164.0, 112.7, 50.9, 38.1, 30.0, 28.8, 28.0, 26.4.

Step 2/3: To a solution of 2-cyclohexylideneacetate (2.11 g, 13.7 mmol, 1.0 eq.) in 56 mL DCM was added DIBAL-H (38.4 mL, 38.4 mmol, 1 M in *c*-Hex) at -78 °C. The reaction mixture was allowed to warm to 0 °C after 3 h. The reaction was quenched with water after 25 h. The mixture was washed with 1 M HCl solution. The aqueous phase was extracted 3x with DCM. The combined organic phases were dried over Na₂SO₄, filtered and the solvent was removed under reduced pressure. The crude was purified via silica pad filtration (2:1 *n*-Hep/EtOAc) to afford 2-cyclohexylideneethan-1-ol as a colorless oil (1.57 g, 12.4 mmol, 91 %). Spectral data are in agreement with literature precedent.^[7]

¹H-NMR (400 MHz, CDCl₃): δ [ppm] = 5.34 (t, *J* = 7.1, 1H), 4.12 (d, *J* = 7.1 Hz, 2H), 2.17 (t, *J* = 5.6 Hz, 2H), 2.10 (t, *J* = 5.7 Hz, 2H), 1.47-1.57 (m, 6H), 1.39 (t, *J* = 6.2 Hz, 1H). **¹³C-NMR** (100 MHz, CDCl₃): δ [ppm] = 144.50, 120.4, 58.6, 37.2, 29.0, 28.5, 28.0, 26.81.

Step 3/3: According to the representative general procedure, 2-cyclohexylideneethan-1-ol (500 g, 3.96 mmol) was reacted. The crude was purified via column chromatography on silica gel (*n*-Hep/EtOAc 3:1; R_f = 0.44 (1:1 *n*-Hep/EtOAc)) to yield **1f** as a white solid (530 mg g, 2.87 mmol, 185.22 g/mol, 73 %). Spectral data are in agreement with literature precedent.^[3]

¹H-NMR (400 MHz, CDCl₃): δ [ppm] = 7.28 (s, 1H, -NH-), 7.13 (s, 1H, -OH), 5.28 (t, *J* = 7.4 Hz, 1H), 4.66 (d, *J* = 7.4 Hz, 2H), 2.19 (s, 2H), 2.11 (s, 2H), 1.55 (s, 6H). **¹³C-NMR** (100 MHz, CDCl₃): δ [ppm] = 159.6, 148.0, 114.6, 62.4, 37.0, 29.0, 28.3, 27.7, 26.6. **FT-IR** (ATR) ν [cm⁻¹] = 3267 (w), 2928 (w), 1714 (m), 1442 (m), 1258 (m), 1104 (s). **Melting point:** 67.0 °C.

S-(4-(prop-1-en-2-yl)cyclohex-1-en-1-yl)methyl N-hydroxycarbamate (**1g**)

According to the representative general procedure, S-(–)-perillyl alcohol (0.5 mL, 3.2 mmol) was reacted. The crude was purified via column chromatography on silica gel (*n*-Hep/EtOAc 4:1 → 2:1; R_f = 0.56 (1:1 *n*-Hep/EtOAc)) to yield **1g** as a white solid (632 mg g, 3.0 mmol, 211.26 g/mol, 95 %).

¹H-NMR (400 MHz, CDCl₃): δ [ppm] = 7.27 (s, 1H, -NH-), 6.90 (s, 1H, -OH), 5.78 (s, 1H), 4.72 (d, *J* = 10.2 Hz, 2H), 4.54 (s, 2H), 2.22-1.80 (m, 6H), 1.73 (s, 3H), 1.54-1.41 (m, 1H). **¹³C-NMR** (100 MHz, CDCl₃): δ [ppm] = 159.5, 149.5, 132.3, 126.7, 108.9, 70.2, 40.7, 30.4, 27.2, 26.3, 20.8. **FT-IR** (ATR) ν [cm⁻¹] = 3321 (vw), 3231 (vw), 2916 (vw), 1694 (s), 1518 (m), 1280 (m), 1118 (s). **Melting point:** 103.0 °C. **HRMS** (ESI): *m/z* [M+Na⁺] calcd for C₁₁H₁₇NO₃: 234.1101; found: 234.1105.

4,6,6-trimethylbicyclo[3.1.1]hept-3-en-2-yl *N*-hydroxycarbamate (**1h**)

To a solution of verbenone (300 mg, 2 mmol, 1.0 eq) in 2 mL Et₂O was added lithium aluminium hydride (2 mmol, 1.0 eq.) at -78 °C. After stirring for 30 min, the reaction mixture was allowed to warm to 0 °C. After 3 h the mixture was quenched with H₂O. The phases were separated and the aqueous phase was extracted 2x with Et₂O. The combined organic phase were dried over Na₂SO₄, filtered and the solvent was removed under reduced pressure. The crude was used for the next step after a silica-pad filtration (EtOAc). According to the representative general procedure, crude Verbenol was reacted. The crude was purified via column chromatography on silica gel (*n*-Hep/EtOAc 4:1 → 2:1; *R_f* = 0.41 (1:1 *n*-Hep/EtOAc)) to yield **1h** as a colorless oil (221 mg, 1.15 mmol, 220.8 g/mol, 52 %).

¹H-NMR (400 MHz, CDCl₃): δ [ppm] = 7.15 (s, 1H), 6.74 (bs, 1H), 5.55-5.49 (m, 1H), 5.41-5.34 (m, 1H), 2.55-2.48 (m, 1H), 2.45-2.38 (m, 1H), 2.03 (t, *J* = 5.4 Hz, 1H), 1.77 (t, *J* = 1.6 Hz, 3H), 1.44-1.40 (m, 1H), 1.36 (s, 3H), 1.00 (s, 3H). **¹³C-NMR** (100 MHz, CDCl₃): δ [ppm] = 159.4, 150.3, 115.2, 77.7, 47.5, 45.6, 39.7, 35.7, 26.6, 22.7, 22.6. **FT-IR** (ATR) ν [cm⁻¹] = 3291 (vw), 2926 (w), 1712 (m), 1442 (w), 1249 (m), 1103 (s). **HRMS** (ESI): *m/z* [M+H⁺] calcd for C₁₁H₁₇NO₃: 212.1282; found: 212.1283.

Z-3-phenylbut-2-en-1-yl *N*-hydroxycarbamate (**1i**)

Step 1/3: To a suspension of CuI (0.43 g, 0.22 mmol, 0.05 eq.) and LiCl (0.19 g, 0.45 mmol, 0.1 eq.) in 34 mL THF was stirred for 30 min at -78 °C. Ethylbut-2-ynoate (0.5 g, 4.46 mmol, 1.0 eq.) and TMSOTf (1.04 g, 4.69 mmol, 1.05 eq.) were added and stirred for additional 5 min followed by a dropwise addition of PhMgBr (0.91 g, 5.45 mmol, 1.2 eq.). The reaction was followed by TLC and TFA (0.61 g, 5.35 mmol, 1.2 eq.) was added after full conversion of the substrate. The organic phase was washed with saturated NaHCO₃ solution. The combined aqueous phases were extracted 3x with EtOAc. The combined organic phases were washed with water and brine, dried over Na₂SO₄, filtered and the solvent was removed under reduced pressure. The crude was purified via column chromatography (19:1 *n*-Pen/Et₂O) to yield ethyl *E*-3-phenylbut-2-enoate (0.11 g, 0.58 mmol, 13 %) and ethyl *Z*-3-phenylbut-2-enoate (0.28 g, 1.47 mmol, 33 %) as colorless oils. Spectral data are in agreement with literature precedent.^[14]

Z-Isomer: **¹H NMR** (400 MHz, CDCl₃): δ [ppm] = 7.38-7.30 (m, 3H), 7.23-7.18 (m, 2H), 5.92 (q, *J* = 1.4 Hz, 1H), 4.00 (q, *J* = 7.1 Hz, 2H), 2.18 (d, *J* = 1.5 Hz, 3H), 1.08 (t, *J* = 7.1 Hz, 3H). **¹³C NMR** (100 MHz, CDCl₃): δ [ppm] = 166.0, 155.4, 140.9, 127.9, 127.7, 126.8, 117.8, 59.7, 27.2, 14.0.

E-Isomer: **¹H-NMR** (400 MHz, CDCl₃): δ [ppm] = 7.50-7.46 (m, 2H), 7.41-7.34 (m, 3H), 6.14 (q, *J* = 1.3 Hz, 1H), 4.22 (q, *J* = 7.1 Hz, 1H), 2.58 (d, *J* = 1.4 Hz, 3H), 1.32 (t, *J* = 7.0 Hz, 3H). **¹³C-NMR** (100 MHz, CDCl₃): δ [ppm] = 167, 155.6, 142.4, 129.1, 128.6, 126.4, 117.3, 60.0, 18.1, 14.5.

Step 2/3: To a solution of ethyl Z-3-phenylbut-2-enoate (0.52 g, 2.72 mmol, 1.0 eq.) in 11 mL DCM was added DIBAL-H (7.7 mL, 7.7 mmol, 2.8 eq., 1 M in *c*-Hex) at -78 °C. After 1.5 h the reaction mixture was allowed to warm 0 °C and quenched with EtOAc and water. The mixture was washed with 1 M HCl solution. The phases were separated and the aqueous phase was extracted 3x with DCM. The combined organic phases were dried over Na₂SO₄, filtered and the solvent was removed under reduced pressure. The crude was purified via column chromatography (2:1 *n*-Hep/EtOAc) to yield Z-3-phenylbut-2-en-1-ol (0.37 g, 2.59 mmol, 96 %) as a colorless oil. Spectral data are in agreement with literature precedent.^[15]

¹H-NMR (400 MHz, CDCl₃): δ [ppm] = 7.39-7.24 (m, 3H), 7.20-7.14 (m, 2H), 5.71 (tq, *J* = 7.0, 1.3 Hz, 1H), 4.07 (d, *J* = 7.0 Hz, 2H), 2.09 (dd, *J* = 2.4, 1.0 Hz, 3H). **¹³C-NMR** (100 MHz, CDCl₃): δ [ppm] = 140.8, 140.2, 128.2, 127.8, 127.2, 126.1, 60.3, 25.4.

Step 3/3: According to the representative general procedure, Z-3-phenylbut-2-en-1-ol (0.32 g, 2.16 mmol) was reacted. The crude was purified via column chromatography on silica gel (*n*-Hep/EtOAc 4:1 → 2:1; *R*_f = 0.18 (2:1 *n*-Hep/EtOAc)) to yield **1i** as a white solid (0.38 g, 1.86 mmol, 207.23 g/mol, 86 %).^[19]

¹H-NMR (400 MHz, CDCl₃): δ [ppm] = 7.40-7.27 (m, 3H, -Ph), 7.21-7.14 (m, 2H, -Ph), 7.12 (s, 1H, -NH-), 6.20 (s, 1H, -OH), 5.67 (td, *J* = 7.2, 1.5 Hz, 1H), 4.59 (dd, *J* = 7.2, 0.9 Hz, 2H), 2.10 (d, *J* = 1.3 Hz, 3H). **¹³C-NMR** (100 MHz, CDCl₃): δ [ppm] = 159.2, 143.7, 140.2, 128.3, 127.7, 127.5, 120.5, 64.1, 25.5. **FT-IR** (ATR) ν [cm⁻¹] = 3298 (w), 1703 (m), 1482 (m), 1266 (m), 1126 (s), 761 (s). **Melting point:** 61.6 °C.

***E*-hex-2-en-1-yl N-hydroxycarbamate (*E*-1j)**

According to the representative general procedure, *E*-hex-2-en-1-ol (1.0 g, 9.98 mmol) was reacted. The crude was purified via column chromatography on silica gel (*n*-Hep/EtOAc 3:1; *R*_f = 0.36 (1:1 *n*-Hep/EtOAc)) to yield ***E*-1j** as a colorless oil (1.07 g, 6.73 mmol, 159.19 g/mol, 67 %). Spectral data are in agreement with literature precedent.^[1]

¹H-NMR (400 MHz, CDCl₃): δ [ppm] = 7.35 (s, 1H, -NH-), 7.19 (s, 1H, -OH), 5.86-5.76 (m, 1H), 5.63-5.53 (m, 1H), 4.61 (d, *J* = 6.6 Hz, 2H), 2.05 (q, *J* = 7.0 Hz, 2H), 1.49-1.37 (m, 2H), 0.92 (t, *J* = 7.4 Hz, 3H). **¹³C-NMR** (100 MHz, CDCl₃): δ [ppm] = 159.4, 137.3, 123.5, 67.0, 34.3, 22.0, 13.6. **FT-IR** (ATR) ν [cm⁻¹] = 3300 (w), 2958 (w), 1707 (m), 1457 (w), 1260 (m), 1110 (s).

***Z*-hex-2-en-1-yl N-hydroxycarbamate (*Z*-1j)**

According to the representative general procedure, *Z*-hex-2-en-1-ol (1.0 g, 9.98 mmol) was reacted. The crude was purified via column chromatography on silica gel (3:1 *n*-Hep/EtOAc; *R*_f = 0.2 (1:1 *n*-Hep/EtOAc)) to yield ***Z*-1j** as a colorless oil (1.28 g, 8.75 mmol, 159.19 g/mol, 88 %). Spectral data are in agreement with literature precedent.^[1]

¹H-NMR (400 MHz, CDCl₃): δ [ppm] = 7.23 (s, 1H, -NH-), 6.79 (s, 1H, -OH), 5.72-5.62 (m, 1H), 5.58-5.49 (m, 1H), 4.71 (d, *J* = 6.9 Hz, 2H), 2.08 (q, *J* = 7.3 Hz, 2H), 1.47-1.34 (m, 2H), 0.91 (t, *J* = 7.4 Hz, 3H). **¹³C-NMR** (100 MHz, CDCl₃): δ [ppm] = 159.5, 135.9, 122.9, 62.0, 29.5, 22.5, 13.6. **FT-IR** (ATR) ν [cm⁻¹] = 3294 (w), 2960 (w), 1706 (m), 1458 (w), 1259 (m), 1108 (s).

4-methylenehex-5-en-1-yl *N*-hydroxycarbamate (**1k**)

4-methylenehex-5-en-1-ol was prepared from myrcene according to literature precedent.^[10] According to the representative general procedure, freshly prepared 4-methylenehex-5-en-1-ol (50 mg, 0.45 mmol) was reacted. The crude was purified via column chromatography on silica gel (5:1 *n*-Hep/EtOAc; *R*_f = 0.34 (1:1 *n*-Hep/EtOAc)) to yield **1k** as a colorless oil (37.4 mg, 0.22 mmol, 171.2 g/mol, 49 %). Spectral data are in agreement with literature precedent.^[11]

¹H-NMR (400 MHz, CDCl₃): δ [ppm] = 7.24 (s, 1H, -NH-), 6.79 (bs, 1H, -OH), 6.37 (dd, *J* = 17.6, 10.8 Hz, 1H), 5.21 (d, *J* = 17.6 Hz, 1H), 5.07 (d, *J* = 11.0 Hz, 1H), 5.02 (d, *J* = 19.2 Hz, 2H), 4.19, (t, *J* = 6.5 Hz, 2H), 2.31-2.25 (m, 2H), 1.8-1.9 (m, 2H). **¹³C-NMR** (100 MHz, CDCl₃): δ [ppm] = 159.5, 145.0, 138.5, 116.3, 113.5, 66.0, 27.4, 27.2. **FT-IR** (ATR) ν [cm⁻¹] = 3279 (w), 2961 (vw), 1712 (m), 1455 (w), 1259 (m), 1112 (s).

N-hydroxy-2-(2-methylprop-1-en-1-yl)benzamide (**1l**)

Step 1/3: A solution of methyl 2-bromobenzoate (1 g, 4.65 mmol, 1.0 eq.), 4,4,5,5-tetramethyl-2-(2-methylprop-1-en-1-yl)-1,3,2-dioxaborolane (1.27 g, 6.98 mmol, 1.5 eq.) and Cs₂CO₃ (3 g, 9.5 mmol, 2.0 eq.) in 11 mL dioxane/H₂O (4:1) was degassed with Argon for 0 min. Pd(dppf)Cl₂·DCM (380 mg, 0.47 mmol, 0.1 eq.) was added and the reaction mixture was heated to 100 °C for 3 h. The mixture was cooled down and filtered through celite using EtOAc. The filtrate was washed with brine, dried over Na₂SO₄, filtered and the solvent was removed under reduced pressure. The crude was purified via column chromatography on silica gel (10:1 *n*-Hep/EtOAc; *R*_f = 0.62 (5:1 *n*-Hep/EtOAc)) to yield methyl 2-(2-methylprop-1-en-1-yl)benzoate (799.2 mg, 4.2 mmol, 190.24 g/mol, 90 %).

¹H-NMR (400 MHz, CDCl₃): δ [ppm] = 7.92-7.87 (m, 1H), 7.45 (td, *J* = 7.6, 1.4 Hz, 1H), 7.26 (t, *J* = 7.4 Hz, 2H), 6.66 (s, 1H), 3.87 (s, 3H), 1.93 (d, *J* = 1.2 Hz, 3H), 1.71 (d, *J* = 1.2 Hz, 3H). **¹³C-NMR** (100 MHz, CDCl₃): δ [ppm] = 167.9, 140.1, 134.8, 131.4, 131.3, 130.2, 129.3, 126.0, 124.7, 51.9, 26.3, 19.3.

Step 2/3: To a solution of methyl 2-(2-methylprop-1-en-1-yl)benzoate (546 mg, 2.87 mmol) in 40 mL MeOH was added 22 mL 1M NaOH. The reaction mixture was stirred overnight and was then slightly acidified with diluted HCl. The aqueous phase was extracted 3x with EtOAc. The combined organic

phases were dried over Na₂SO₄, filtered and the solvent was removed under reduced pressure. The crude was purified via silica pad filtration to yield 2-(2-methylprop-1-en-1-yl)benzoic acid (503.9 mg, 2.64 mmol, 191.23 g/mol, 92 %).

¹H-NMR (400 MHz, CDCl₃): δ [ppm] = 8.05 (d, *J* = 7.8 Hz, 1H), 7.53-7.44 (m, 1H), 7.35-7.27 (m, 2H), 6.71 (s, 1H), 1.93 (s, 3H), 1.73 (s, 3H). **¹³C-NMR** (100 MHz, CDCl₃): δ [ppm] = 140.7, 140.1, 135.3, 132.2, 131.5, 131.2, 126.2, 124.7, 26.3, 19.4.

Step 3/3: To a solution of yield 2-(2-methylprop-1-en-1-yl)benzoic acid (200 mg, 1.05 mmol, 1.0 eq.) and a catalytic amount of DMF in 5 mL DCM was added oxalyl chloride (400 mg, 3.15 mmol, 3.0 eq.) at 0 °C. The solvent was removed after 3 h and the residue was again dissolved in 1 mL DCM. NaHCO₃ (198 mg, 2.36 mmol, 2.25 eq.) was dissolved in 1.2 mL H₂O and cooled to 0 °C. Hydroxylamine hydrochloride (91 mg, 1.31 mmol, 1.25 eq.) was added and the mixture was stirred for 30 min. before the residue in DCM was added. The mixture was stirred at 0 °C for 30 min. and was then allowed to warm to room temperature. After 2 h, EtOAc and H₂O was added to the reaction mixture and the aqueous phase was extracted 2x with EtOAc. The combined organic phases were washed with brine, dried over Na₂SO₄, filtered and the solvent was removed under reduced pressure. The crude was purified via column chromatography on silica gel (5:1 → 1:1 *n*-Hep/EtOAc; R_f = 0.18 (1:1 *n*-Hep/EtOAc)) to yield **11** as a white solid (126.1 mg, 0.66 mmol, 191.23 g/mol, 63 %).

¹H-NMR (400 MHz, CDCl₃): δ [ppm] = 8.94 (s, 2H), 7.81 (d, *J* = 5.4 Hz, 1H), 7.46 (t, *J* = 7.5 Hz, 1H), 7.33 (t, *J* = 7.4 Hz, 1H), 7.22 (d, *J* = 7.6 Hz, 1H), 6.44 (s, 1H), 1.95 (s, 3H), 1.75 (s, 3H). **¹³C-NMR** (100 MHz, CDCl₃): δ [ppm] = 139.3, 136.6, 131.0, 131.0, 130.8, 129.1, 126.9, 123.1, 26.1, 19.4. **FT-IR** (ATR) ν [cm⁻¹] = 3303 (w), 2906 (vw), 1633 (m), 1514 (m), 1019 (m). **Melting point:** 129.9 °C. **HRMS** (ESI): *m/z* [M+H⁺] calcd for C₁₁H₁₃NO₂: 192.1019; found: 192.1030.

2.2 GOx/HRP mediated intramolecular nitroso ene reactions

General procedure with GOx/HRP: To a 10 mM solution of substrate in 7 mL phosphate buffer (100 mM, pH 7.0), if necessary including 10 vol% of desired cosolvent (dioxane or EtOAc), was added 70 U horseradish peroxidase and 70 U glucose oxidase (*Aspergillus Niger*). D-glucose (50 mM) was added and the reaction was incubated at 25 °C. After full conversion of starting material (followed by TLC), the reaction mixture was extracted 3x with EtOAc. The combined organic phases were dried over Na₂SO₄, filtered and the solvent was removed under reduced pressure. The crude was purified via silica pad filtration or flash column chromatography on silica gel.

3-Hydroxy-4-(prop-1-en-2-yl)oxazolidin-2-one (**2a**)

According to the general procedure with GOx/HRP, **1a** was reacted and full conversion was observed after 2 h. The crude was purified via silica pad filtration with EtOAc to yield **2a** as colorless crystals (9.7 mg, 0.068 mmol, 143.14 g/mol, 97 %). Spectral data are in agreement with literature precedent.^[2]

¹H-NMR (400 MHz, CDCl₃): δ 8.29 (bs, 1H), 5.10 (dd, *J* = 8.4, 7.1 Hz, 2H), 4.49-4.30 (m, 2H), 4.08-3.97 (m, 1H), 1.81-1.76 (m, 3H). **¹³C-NMR** (100 MHz, CDCl₃): δ 160.9, 139.0, 117.0, 65.7, 64.8, 16.9. **FT-IR** (ATR) ν [cm⁻¹] = 3236 (w), 2923 (w), 1743 (s), 1215 (m), 1095 (m). **Melting point:** 73.0 °C.

3-Hydroxy-4-(d₅-prop-1-en-2-yl)oxazolidin-2-one (**2a-d₅**)

According to the general procedure with GOx/HRP, **1a-d₆** was reacted and full conversion was observed after 2 h. The crude was purified via silica pad filtration with EtOAc to yield **2a-d₅** as colorless crystals (9.4 mg, 0.063 mmol, 148.17 g/mol, 91 %, D₅ ≥ 99 %).

¹H-NMR (400 MHz, CDCl₃): δ [ppm] = 8.26 (bs, 1H, -OH), 4.46-4.36 (m, 2H), 4.07-3.97 (m, 1H). **FT-IR** (ATR) ν [cm⁻¹] = 3183 (w), 2924 (vw), 1748 (s), 1103 (s), 1007 (m). **Melting point:** 67.5 °C. **HRMS** (ESI): *m/z* [M+Na⁺] calcd for C₆H₄D₅NO₃: 171.0788; found: 171.0801.

3-Hydroxy-4-(1,1-d₂-prop-1-en-2-yl)oxazolidin-2-one (2a-d₂)

According to the general procedure with GOx/HRP, *E*-1a-d₃ was reacted and full conversion was observed after 2 h. The crude was purified via silica pad filtration with EtOAc to yield **2a-d₂** (9.5 mg, 0.065 mmol, 145.15 g/mol, 93 %, D₂ ≥ 95 %).

¹H-NMR (400 MHz, CDCl₃): δ [ppm] = 8.17 (s, 1H), 4.46-4.37 (m, 2H), 4.07-3.97 (m, 1H), 1.78 (s, 3H). **FT-IR** (ATR) ν [cm⁻¹] = 3259 (vw), 2917 (vw), 1745 (s), 1212 (m), 1088 (m). **HRMS** (ESI): *m/z* [M+H⁺] calcd for C₆H₇D₂NO₃: 146.0781; found: 146.0784.

3-Hydroxy-4-(3,3,3-d₃-prop-1-en-2-yl)oxazolidin-2-one (2a-d₃)

According to the general procedure with GOx/HRP, *Z*-1a-d₃ was reacted and full conversion was observed after 2 h. The crude was purified via silica pad filtration with EtOAc to yield **2a-d₃** (9.1 mg, 0.062 mmol, 146.15 g/mol, 89 %, D₃ ≥ 95 %).

¹H-NMR (400 MHz, CDCl₃): δ [ppm] = 8.25 (s, 1H), 5.13 (s, 1H), 5.10 (d, *J* = 1.0 Hz, 1H), 4.45-4.37 (m, 2H), 4.05-3.98 (m, 1H). **FT-IR** (ATR) ν [cm⁻¹] = 3259 (vw), 2917 (vw), 1747 (s), 1212 (m), 1089 (m). **HRMS** (ESI): *m/z* [M+Na⁺] calcd for C₆H₆D₃NO₃: 147.0844; found: 146.0842.

1-hydroxy-5-(prop-1-en-2-yl)pyrrolidin-2-one (2b)

According to the general procedure with GOx/HRP, **1b** was reacted in phosphate buffer containing 10 % dioxane and full conversion was observed after 3 h. The crude was purified via column chromatography (DCM + 5 % MeOH + 1 % TEA; R_f = 0.34 (DCM + 5 % MeOH)) to yield **2b** as white solid (7.6 mg, 0.054 mmol, 141.17 g/mol, 77 %). Spectral data are in agreement with literature precedent.^[12]

¹H-NMR (400 MHz, CDCl₃): δ [ppm] = 9.44 (bs, 1H, -OH), 5.00 (d, *J* = 15.5 Hz, 2H), 4.28 (dd, *J* = 8.4, 5.6 Hz, 1H), 2.51-2.31 (m, 2H), 2.29-2.16 (m, 1H), 1.86-1.75 (m, 1H), 1.73 (s, 3H). **¹³C-NMR** (100 MHz, CDCl₃): δ [ppm] = 142.4, 114.2, 65.0, 27.2, 21.5, 16.9. **FT-IR** (ATR) ν [cm⁻¹] = 3085 (vw), 2497 (w), 1668 (s), 1456 (m), 926 (s). **Melting point**: 91.3 °C.

3-hydroxy-4-vinyloxazolidin-2-one (**2c**)

According to the general procedure with GOx/HRP, **1c** was reacted and full conversion was observed after 2 h. The crude was purified via column chromatography (5:1 *n*-Hep/EtOAc; R_f = 0.23 (1:1 *n*-Hep/EtOAc)) to yield **2c** as a white solid with a diastereomeric ratio of 68 % (4.8 mg, 0.037 mmol, 129.12 g/mol, 53 %). Spectral data are in agreement with literature precedent.^[2]

¹H-NMR (400 MHz, CDCl₃): δ [ppm] = 6.78 (bs, 1H, -OH), 5.81 (ddd, J = 17.2, 10.2, 7.8 Hz, 1H), 5.47 (dd, J = 23.3, 13.6 Hz, 2H), 4.43 (t, J = 8.2 Hz, 1H), 4.33 (dd, J = 16.7, 8.2 Hz, H-1), 3.99 (t, J = 8.2 Hz, 1H). **¹³C-NMR** (100 MHz, CDCl₃): δ [ppm] = 160.5, 132.3, 122.3, 66.3, 62.8. **FT-IR** (ATR) ν [cm⁻¹] = 3244 (w), 2922 (vw), 1726 (s), 1471 (w), 1090 (m). **Melting point**: 55.0 °C.

3-hydroxy-4-methyl-4-(prop-1-en-2-yl)oxazolidin-2-one (**2d**)

According to the general procedure with GOx/HRP, **1d** was reacted and full conversion was observed after 2 h. The crude was purified via silica pad filtration with EtOAc to yield **2d** as colorless oil (9.1 mg, 0.064 mmol, 143.14 g/mol, 91 %).

¹H-NMR (400 MHz, CDCl₃): δ [ppm] = 7.65 (bs, 1H, -OH), 5.91 (dd, J = 17.5, 10.8 Hz, 1H), 5.45-5.19 (m, 2H), 4.12 (d, J = 8.4 Hz, 1H), 4.06 (d, J = 8.4 Hz, 1H), 1.47 (d, J = 5.5 Hz, 3H). **¹³C-NMR** (100 MHz, CDCl₃): δ [ppm] = 159.7, 136.6, 118.1, 72.6, 64.1, 18.9. **FT-IR** (ATR) ν [cm⁻¹] = 3260 (w), 2976 (vw), 1746 (s), 1101 (m), 990 (m). **HRMS** (ESI): m/z [M+H⁺] calcd for C₆H₉NO₃: 144.0656; found: 144.0657.

3-hydroxy-5-methyl-4-vinyloxazolidin-2-one (**2e**)

According to the general procedure with GOx/HRP, **1e** was reacted in phosphate buffer containing 10 % EtOAc and full conversion was observed after 5 h. The crude was purified via column chromatography (5:1 *n*-Hep/EtOAc; R_f = 0.29 & 0.21 (1:1 *n*-Hep/EtOAc)) to yield **2e** colorless oil with a diastereomeric ratio of 68 % (5.2 mg, 0.036 mmol, 143.14 g/mol, 52 %). Spectral data are in agreement with literature precedent.^[5]

Major diastereomer: **¹H-NMR** (400 MHz, CDCl₃): δ [ppm] = 7.80 (bs, 1H, -OH), 5.87-5.71 (m, 1H), 5.53-5.38 (m, 2H), 4.75-4.65 (m, 1H), 4.33 (t, J = 8.0 Hz, 1H), 1.32 (d, J = 6.6 Hz, 3H). **¹³C-NMR** (100 MHz, CDCl₃): δ [ppm] = 130.1, 122.9, 75.1, 65.8, 16.0.

Minor diastereomer (selected signals): **¹H-NMR** (400 MHz, CDCl₃): δ [ppm] = 4.22 (dq, *J* = 9.3, 6.2 Hz, 1H), 3.92-3.81 (m, 1H), 1.44 (d, *J* = 6.2 Hz, 3H). **¹³C-NMR** (100 MHz, CDCl₃): δ [ppm] = 132.4, 122.5, 73.4, 70.3, 17.8.

FT-IR (ATR) ν [cm⁻¹] = 3280 (vw), 2936 (vw), 1748 (s), 1220 (m), 1057 (m).

4-(cyclohex-1-en-1-yl)-3-hydroxyoxazolidin-2-one (**2f**)

According to the general procedure with GOx/HRP, **1f** was reacted in phosphate buffer containing 10 % EtOAc and full conversion was observed after 2 h. The crude was purified via column chromatography (4:1 *n*-Hep/EtOAc; *R_f* = 0.35 (1:1 *n*-Hep/EtOAc)) to yield **2f** as colorless crystals (9.9 mg, 0.054 mmol, 183.21 g/mol, 77 %). Spectral data are in agreement with literature precedent.^[5]

¹H-NMR (400 MHz, CDCl₃): δ [ppm] = 8.09 (bs, 1H, -OH), 5.83 (s, 1H), 4.38-4.28 (m, 2H), 4.08-3.97 (m, 1H), 2.14-1.86 (m, 4H), 1.72-1.50 (m, 4H). **¹³C-NMR** (100 MHz, CDCl₃): δ [ppm] = 160.9, 131.6, 129.4, 65.7, 65.4, 25.1, 22.8, 22.2, 22.1. **FT-IR** (ATR) ν [cm⁻¹] = 3252 (vw), 2927 (w), 1741 (s), 1096 (m), 1016 (m). **Melting point**: 90.1 °C.

8*R*-1-hydroxy-8-(prop-1-en-2-yl)-3-oxa-1-azaspiro[4.5]dec-6-en-2-one (**2g**)

According to the general procedure with GOx/HRP, **1g** was reacted in phosphate buffer containing 10 % EtOAc and full conversion was observed after 4 h. The crude was purified via column chromatography (3:1 *n*-Hep/EtOAc; *R_f* = 0.44 (1:1 *n*-Hep/EtOAc)) to yield **2g** as a colorless oil with diastereomeric ratio of 92 % (14.2 mg, 0.068 mmol, 209.25 g/mol, 97 %).

Major diastereomer: **¹H-NMR** (400 MHz, CDCl₃): δ [ppm] = 8.03 (bs, 1H, -OH), 5.99 (dd, *J* = 10.0, 3.8 Hz, 1H), 5.67 (dd, *J* = 10.1, 1.5 Hz, 1H), 4.82 (d, *J* = 34.0 Hz, 2H), 4.11-3.99 (m, 2H), 2.68 (d, *J* = 4.9 Hz, 1H), 2.30-2.15 (m, 1H), 1.90 (ddd, *J* = 13.0, 8.0, 4.2 Hz, 1H), 1.76 (s, 3H), 1.78-1.68 (m, 1H), 1.64-1.53 (m, 1H). **¹³C-NMR** (100 MHz, CDCl₃): δ [ppm] = 159.5, 145.7, 137.8, 126.2, 112.7, 71.7, 62.3, 41.3, 25.9, 23.4, 21.7.

Minor diastereomer (selected signals): **¹H-NMR** (400 MHz, CDCl₃): δ [ppm] = 6.09 (d, *J* = 10.2 Hz, 1H), 5.59 (d, *J* = 9.3 Hz, 1H), 2.86-2.79 (m, 1H). **¹³C-NMR** (100 MHz, CDCl₃): δ [ppm] = 147.2, 138.4, 126.6, 111.0, 71.5, 63.0, 42.5, 26.9, 25.2, 20.7.

FT-IR (ATR) ν [cm⁻¹] = 3263 (vw), 2935 (vw), 1748 (s), 1451 (w), 1088 (m). **HRMS** (ESI): m/z [M+H⁺] calcd for C₁₁H₁₅NO₃: 210.1125, found: 210.1128.

***E*-4-(but-1-en-1-yl)-3-hydroxyoxazolidin-2-one (2j)**

According to the general procedure with GOx/HRP, ***E*-1j** was reacted in phosphate buffer containing 10 % EtOAc and full conversion was observed after 2 h. The crude was purified via column chromatography (4:1 *n*-Hep/EtOAc; R_f = 0.34 (1:1 *n*-Hep/EtOAc)) to yield **2j** as a colorless oil with *E/Z* ratio of 75:25 (7.3 mg, 0.046 mmol, 157.17 g/mol, 66 %). Spectral data are in agreement with literature precedent.^[2]

E-Isomer: **¹H-NMR** (400 MHz, CDCl₃): δ [ppm] = 7.72 (bs, 1H, -OH), 5.94 (dt, J = 15.3, 6.3 Hz, 1H, 5.38 (ddt, J = 15.4, 8.3, 1.6 Hz, 1H), 4.39 (t, J = 8.1 Hz, 1H), 4.30 (dd, J = 16.8, 8.3 Hz, 1H), 3.95 (t, J = 8.1 Hz, 1H), 2.26-2.04 (m, 2H), 1.02 (t, J = 7.4 Hz, 3H). **¹³C-NMR** (100 MHz, CDCl₃): δ [ppm] = 159.4, 137.3, 123.5, 67.0, 34.3, 22.0, 13.6.

Z-Isomer (selected signals): **¹H-NMR** (400 MHz, CDCl₃): δ [ppm] = 5.84 (dt, J = 10.7, 7.7 Hz, 1H), 4.72 (q, J = 9.1 Hz, 1H), 1.01 (t, J = 7.5 Hz, 3H). **¹³C-NMR** (100 MHz, CDCl₃): δ [ppm] = 66.6, 22.5, 14.0.

FT-IR (ATR) ν [cm⁻¹] = 3265 (vw), 2965 (vw), 1754 (s), 1462 (w), 1210 (w), 1081 (m).

2-hydroxy-3-(2-hydroxypropan-2-yl)isoindolin-1-one (7)

According to the general procedure with GOx/HRP, **1I** (was reacted in phosphate buffer containing 10 % EtOAc. The reaction mixture was extracted after 30 h. The crude of four reactions was combined and purified via column chromatography (1:1 *n*-Hep/EtOAc; R_f = 0.11 (1:1 *n*-Hep/EtOAc)) to yield **7** as a white solid (7.7 mg, 0.041 mmol, 207.23 g/mol, 13 %) and reisolate **1I** (33.2 mg, 0.174 mmol, 62 %).

¹H-NMR (400 MHz, CD₃OD): δ [ppm] = 7.86 (d, J = 7.7 Hz, 1H), 7.74 (d, J = 7.5 Hz, 1H), 7.61 (td, J = 7.6, 1.2 Hz, 1H), 7.51 (t, J = 7.5 Hz, 1H), 5.02 (s, 1H), 1.62 (s, 3H), 0.78 (s, 3H). **¹³C-NMR** (100 MHz, CD₃OD): δ [ppm] = 168.4, 142.0, 133.0, 131.5, 129.6, 126.9, 123.6, 84.0, 67.5, 24.6, 19.2.

HRMS (ESI): m/z [M+H⁺] calcd for C₁₁H₁₄NO₃: 208.0969, found: 208.0968.

m/z [M+Na⁺] calcd for C₁₁H₁₃NO₃Na: 230.0787, found: 230.0789.

m/z [M-*i*PrOH+H⁺] calcd for C₈H₆NO₂: 148.0394, found: 148.0394.

m/z [M-*i*PrOH+Na⁺] calcd for C₈H₅NO₂Na: 170.0213, found: 170.0212.

2.3 GOx/HRP mediated nitroso-Diels-Alder reactions

3,10-dioxo-1-azabicyclo[5.3.1]undec-7-en-2-one (6)

According to the general procedure with GOx/HRP, **1k** was reacted and full conversion was observed after 4 h. The crude was purified via column chromatography (3:1 *n*-Hep/EtOAc: R_f = 0.45 (1:1 *n*-Hep/EtOAc)) to yield **6** as colorless crystals (4.3 mg, 0.026 mmol, 169.18 g/mol, 36 %). Spectral data are in agreement with literature precedent.^[13]

¹H-NMR (400 MHz, CDCl₃): δ [ppm] = 5.79-5.74 (m, 1H), 4.73 (dd, J = 14.3, 5.5 Hz, 1H), 4.44-4.33 (m, 2H), 2.20 (dd, J = 15.0, 2.0 Hz, 1H), 4.15-4.04 (m, 1H), 3.49 (d, J = 15.0 Hz, 1H), 2.56-2.39 (m, 1H), 2.35-2.16 (m, 2H), 1.97-1.84 (m, 1H). **¹³C-NMR** (100 MHz, CDCl₃): δ [ppm] = 164.2, 142.1, 123.0, 71.8, 67.3, 52.6, 31.8, 27.4. **FT-IR** (ATR) ν [cm⁻¹] = 2928 (vw), 1742 (s), 1443 (w), 1159 (s), 1050 (s). **Melting point**: 38.0 °C.

2.4 GOx/HRP mediated intermolecular nitroso ene reaction

Benzyl (2,3-dimethylbut-3-en-2-yl)(hydroxy)carbamate (5)

To a 10 mM solution of **3** in 7 mL phosphate buffer (100 mM, pH 7.0) including 10 vol% of EtOAc was added 2,3-dimethylbut-2-ene. 70 U horseradish peroxidase and 70 U glucose oxidase (*Aspergillus Niger*). D-glucose (50 mM) was added and the reaction was incubated at 25 °C. After full conversion of starting material (followed by TLC), the reaction mixture was extracted 3 x with EtOAc. The combined organic phases were dried over Na₂SO₄, filtered and the solvent was removed under reduced pressure. The crude was purified via flash column chromatography on silica gel (12:1 *n*-Hep/EtOAc; R_f = 0.68 (1:1 *n*-Hep/EtOAc)) to afford **5**. Spectral data are in agreement with literature precedent.^[2]

¹H-NMR (400 MHz, CDCl₃): δ [ppm] = 7.37-7.30 (m, 5H), 6.23 (bs, 1H), 5.17 (s, 2H), 4.85 (s, 1H), 4.77 (s, 1H), 1.75 (s, 3H), 1.48 (s, 6H). **¹³C-NMR** (100 MHz, CDCl₃): δ [ppm] = 158.1, 149.5, 135.8, 128.5, 128.3, 128.2, 109.4, 68.0, 66.0, 25.4, 19.1. **FT-IR** (ATR) ν [cm⁻¹] = 3298 (vw), 2925 (vw), 1681 (m), 1326 (m), 1096 (m).

Table S1. Screening of 2,3-dimethylbut-2-ene loading.

Entry	2,3-dimethylbut-2-ene	yield [%] ^[a]
1	1.0 eq.	30
2	1.5 eq.	51
3	5.0 eq.	55
4	as cosolvent ^[b]	86

[a] isolated yield. [b] 10 vol %; instead of EtOAc.

3. Peroxidase Screening

General procedure for peroxidase screenings: To a 10 mM solution of **1a** in 7 mL phosphate buffer (100 mM, pH 7.0), was added 70 U peroxidase and 70 U glucose oxidase (*Aspergillus Niger*). D-glucose (50 mM) was added and the reaction was incubated at 25 °C. The conversion was followed by TLC. After full conversion, the reaction mixture was extracted 3 x with EtOAc. The combined organic phases were dried over Na₂SO₄, filtered and the solvent was removed under reduced pressure. The crude was purified via silica pad filtration or flash column chromatography on silica gel. Reactions with lactoperoxidase and chloroperoxidase were performed in citrate buffer (100 mM, pH 5.5).

Figure S1. Screening of different peroxidases in the nitroso-ene reaction of **1a**. [*] Conversion; yield not determined.

Figure S2. Screening of different substrate loadings in the nitroso-ene reaction of **1a**.

Figure S3. Screening of different GOx loadings at in the nitroso ene reaction of **1a**.

4. Laccase Screening

General procedure for laccase screenings: To a 10 mM solution of **1a** in 7 mL buffer was added 100 U laccase and the reaction was incubated at 20 °C. Full conversion of starting material was followed by TLC, the reaction mixture was extracted 3 x with EtOAc. The combined organic phases were dried over Na₂SO₄, filtered and the solvent was removed under reduced pressure. The crude was purified via flash column chromatography (3:1 *n*-Hep/EtOAc) on silica gel to afford **2a**.

Figure S4. Screening of different laccases in the nitroso-ene reaction of **1a**. [*] no conversion.

5. Kinetic isotope studies

Two reaction sets were run with **1a** and **1a-d₆** to calculate the kinetic isotope effect (KIE). To a 10 mM solution of substrate in 7 mL phosphate buffer (100 mM, pH 7.0) was added 3.5 U horseradish peroxidase and 3.5 U glucose oxidase (*Aspergillus Niger*). D-glucose (6.25 mM) was added and the reaction was incubated at 25 °C. The reaction was stopped at different times (5, 10, 15, 20, 25, 30 min) and worked up as followed. The reaction mixture was extracted 3 x with EtOAc. The combined organic phases were dried over Na₂SO₄, filtered through a silica pad and the solvent was removed under reduced pressure. The conversion was determined by ¹H-NMR analysis, from which the KIE was calculated. Both reaction sets were performed twice under the same conditions. The overall average KIE was determined as inverse β secondary kinetic isotope effect $k_H/k_D = 0.79 \pm 0.04$.

Table S2. Results of kinetic measurements of **1a** and **1a-d₆**.

Entry	time [min]	conversion [%] ^[a]				k _H /k _D		
		1a	1a ^[b]	1a-d₆	1a-d₆ ^[b]	1 st set	2 nd set	averaged
1	5	4.0	3.8	6.9	5.8	0.57	0.66	0.61 ± 0.05
2	10	7.6	8.4	9.2	10.9	0.82	0.77	0.8 ± 0.03
3	15	11.8	14.1	13.1	14.2	0.9	0.99	0.95 ± 0.05
4	20	13.0	16.8	19.3	21.0	0.68	0.8	0.74 ± 0.06
5	25	16.5	18.4	22.7	26.3	0.73	0.7	0.71 ± 0.02
6	30	23.9	25.0	27.4	26.3	0.87	0.95	0.91 ± 0.04

[a] Determined by ¹H-NMR analysis. [b] Second reaction set with identical conditions.

Figure S7. initial rates for the HRP-mediated conversions of **1a** and **1a-d₆**.

6. NMR Spectra

7. References

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