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Review

Bromoperoxidases and functional enzyme mimics as catalysts for oxidative bromination—A sustainable synthetic approach

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Dedicated to Prof. Dr. Bernd Giese on the occasion of his 70th birthday.

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ABSTRACT

The discovery of enzymes that utilize hydrogen peroxide to oxidize bromide under physiological conditions provided a strong stimulus to the field of oxidative bromination. A synthetically useful enzyme, to catalyze the oxidation of bromide, for bromofunctionalization of donor-substituted arenes in solutions of hydrogen peroxide and sodium bromide, is a vanadate(V)-dependent bromoperoxidase from the brown alga *Ascophyllum nodosum*. This enzyme operates in homogeneous solutions of buffered aqueous *tert*-butanol (pH 6.2), or, to simplify repetitive use, in a two-phase system after immobilization onto magnetic beads. Synthesis of cyclic bromohydrin ethers (tetrahydrofurans and tetrahydropyrans) and vicinal dibromides from unsaturated hydrocarbons, on the other hand, occurs more effectively in polar aprotic solvents. Under such conditions the more lipophilic *tert*-butyl hydroperoxide serves as oxidant, which is activated by oxovanadium(V) complexes (functional bromoperoxidase mimics). Protons and bromide ions, which are consumed for in situ generation of bromine, are supplied in organic solution by fragmentation of 3-bromopropionic acids. The structure-reactivity data obtained from oxidations catalyzed by bromoperoxidases and functional enzyme mimics pose a valuable guideline for predicting selectivity in biomimetic synthesis of organobromines from terpenes, acetogenins, and pyrrole alkaloids.

Abbreviations: BPO, bromoperoxidase; CHD, cyclohexa-1,4-diene; DTAB, dodecyl trimethyl ammonium bromide; HOMO, highest occupied molecular orbital; LUMO, lowest unoccupied molecular orbital; MES, morpholine-4-ethanesulfonic acid; M-PVA, magnetic polyvinyl alcohol-coated beads, either epoxide (E) or amino-functionalized (N), 01 and 12 refer to particle sizes; ^{118}NBS, *N*-bromosuccinimide; NHE, normal hydrogen electrode; TBHP, *tert*-butyl hydroperoxide; V_{Br}PO, vanadate(V)-dependent bromoperoxidase; V_{Br}PO(*An*I), vanadate(V)-dependent bromoperoxidase from *Ascophyllum nodosum*, isoenzyme I, PDB access code 1QI9.

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1. Introduction

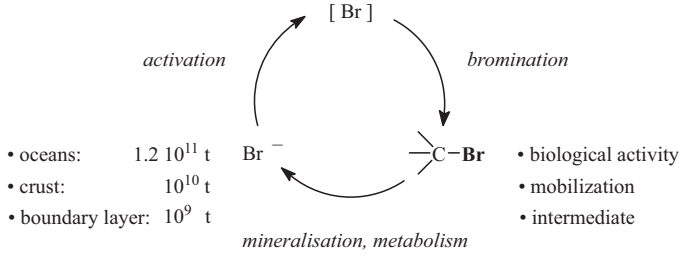
> . . .A myriad of simple haloalkanes have been isolated from marine algae. . .It seems likely that the "smell of the ocean" is due to this potpourri of volatile halocarbons. . .. [1]

Organobromines have always come along with human civilization and will continue to do so [2,3]. Bromoalkanes are formed in enormous quantity and diversity from photochemical reactions in the marine boundary layer [4,5], geothermal events [6], and metabolic pathways (Scheme 1) [7–9]. Industrial production of organobromines started in the middle of the 19th century [10], that is, with some offset from the time that Carl Löwig (1825) [11,12] and Antoine-Jérôme Balard (1826) discovered bromine as a new chemical element [13,14].

The use of organobromines in life and society is a tribute to the unique properties of the carbon–bromine bond. A bromosubstituent is able to raise lipophilicity of an organic molecule, which improves properties of a substance to serve as emulsifier, insecticide, or biologically active compound [15,16]. Aryl-bound bromosubstituents lower the rate of oxidative arene metabolism, which is important to increase the in vivo stability of pharmaceuticals. Conjugation between an aromatic π-system and a pair of non bonding electrons at bromine shifts electronic excitation energies of chromophors and thus induces auxochromic effects. In other arenes, the carbon bromine bond is a key structural element to achieve flame retarding properties of a material (vide infra) [17–20]. The aliphatic carbon–bromine bond, on the other hand, combines in a unique manner stability and chemical reactivity. This bond can be selectively broken in heterolytic or homolytic reactions, to serve as progenitor of carbocations, carbanions, or free carbon radicals [10,21]. The propensity to undergo chemical transformations is the reason for the use of bromoalkanes as rodenticides [22], fire extinguishants [23], and gasoline additives [10]. Surprisingly little is known about the biological role of naturally occurring organobromines [3], but it seems reasonable to assume that producing organisms have similar interests in these substances as man.

The common bromine source for synthesis of organobromines is bromide. Bromide-containing minerals, such as bromocarnallite ($MgBr_2{\cdot}KBr{\cdot}6H_2O$), bromosylvinite [K(Cl,Br)], and silver halide ores {bromoargyrite (AgBr), embolite [Ag(Cl,Br)], capgaronnite [HgS·Ag(Cl,Br)]} unfortunately are rare in the earth's crust [10]. Seemingly unlimited quantities of bromide, however, are dissolved in the oceans (65 mg L^{-1} to 6.5 g L^{-1}), which therefore serve as industrial bromide resource to produce electrophilic bromination agents [10].

In synthesis, carbon–bromine bonds are formed from radical reactions, nucleophilic substitutions, and electrophilic additions [2,24]. The majority of organobromines, however, originates from electrophilic additions, which requires an a priori oxidation of bromide [25,26]. To oxidize bromide on a technical scale, manufacturers in the early days used manganese dioxide–sulfuric acid blends, whereas chlorine (Steaming-Out Process) or air–chlorine mixtures in combination with sulfuric acid (Blowing-Out Process) are standard procedures today. The annual production of bromine has continuously increased over the past decades and has reached a global volume of more than 563,000 ton world wide in 2004 [10].



Scheme 1. Sources and sinks of organobromine compounds in the planetary bromine cycle {[Br] = Br^+_{solv}, Br•, BrO_n•, BrX_n^- (n = 1, 2, . . .; X = e.g. Cl, Br), BrY (Y = Br, OH)}.

Molecular bromine is a favorable agent for synthesis, but it is corrosive and toxic. Transport, storage, and handling of bromine therefore require strict safety standards [10]. For synthesis of 1 ton of the flame retardant tetrabromobisphenol A (Scheme 2), 1.18 ton of bromine are needed. To produce this amount of bromine, bromide from about 134 m^3 of Dead Sea brine [ρ_{20} = 1.2334 g L^{-1}, 0.055 molar in bromide] [27,28] has to be crystallized as alkali salt and subsequently oxidized. Production of 1 ton of tetrabromobisphenol A also provides 165 m^3(0.6 ton), of hydrogen bromide as by-product. The hydrogen bromide must be separated in a recovery unit and disposed of, or oxidatively recycled. Hydrogen bromide, which is either supplied from a waste stream or an alkali bromide–mineral acid mixture, is oxidized on a technical basis with hydrogen peroxide at elevated temperature, to in situ-produce bromine. This method is the chemical basis for a procedure called the *on-site bromination* and is used to industrially prepare bromoarenes [29]. Low world market prices of bromine (1.39 $ per kilogram in 2006 in the United States) [30], high energy costs, and limited fields of application, however, preclude a wider spread use of the on-site bromination concept at the moment.

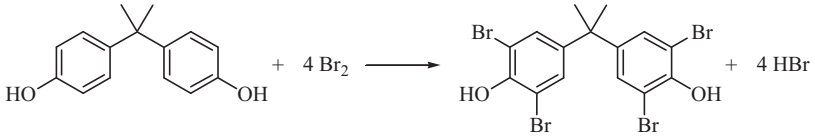
The lessons learned from nature show that the chemical elements are turned over in cycles, and sustainable production of organobromines therefore has to somehow follow the planetary bromine cycle (Scheme 1) [31]. The planetary bromine cycle starts from bromide. By looking at the structures of brominated secondary metabolites it is obvious that in biochemistry, as in organic synthesis, electrophilic hydrocarbon bromination dominates. To perform electrophilic bromination in nature requires the existence of effective pathways for bromide oxidation under physiological conditions. The quest for the origin of naturally produced organohalogens [32–34], led, among others, to the discovery of the marine bromoperoxidases [7,35–37], which utilize hydrogen peroxide to oxidize bromide dissolved in ocean water [38–40].

In the decades that followed the discovery of the bromoperoxidases, oxidative bromination has expanded into a dynamic area of research [25]. In this article, we therefore summarize the most recent developments in bromoperoxidase chemistry, and transformations using functional mimics for sustainable synthesis of organobromines [41,42]. To give the reader a better understanding of vanadate(V)-catalyzed oxidation for synthesis of naturally occurring organobromines, we included aspects of peroxide chemistry of vanadium(V), and peroxide-mediated oxidation of bromide. For bromide oxidation catalyzed by rhenium- [43], molybdenum- [44], tungsten- [39,45], and cobalt complexes [46], as well as organobromine formation via free radical reactions [47,48] or nucleophilic substitutions [49], the reader is referred to specialized articles, reviews, and book chapters [50,51]. The same should be done for aspects dealing with the systematics of brominated secondary metabolites [52–54].

2. General aspects

2.1. Peroxide-mediated oxidation of bromide

Standard reagents to oxidize bromide in bromoperoxidase chemistry are hydrogen peroxide and *tert*-butyl hydroperoxide (TBHP) [55]. For both compounds the mass fraction of transferable and therefore *active* oxygen is significant (47% for hydrogen peroxide and 17.8% for *tert*-butyl hydroperoxide), and by-products that are left from the oxygen atom transfer (water and *tert*-butanol) pose no concern for waste disposal. Hydrogen peroxide is administered



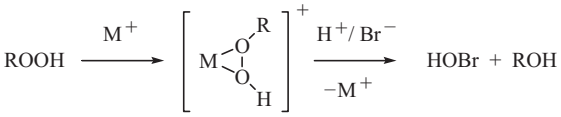
Scheme 2. Stoichiometry for synthesis of tetrabromobisphenol A [10,18].

as aqueous 30–50% solution, crystalline urea hydrogen peroxide (UHP) inclusion compound, or as hydrolyzable persalt (percarbonate, perborate or persulfate) [56]. *tert*-Butyl hydroperoxide is commercially available as 5.5 molar solution in nonane, 80% solution in di-*tert*-butylperoxide and water, and a reasonably priced 70% aqueous solution. The aqueous solution may be used to prepare an anhydrous ~3 molar solution of *tert*-butyl hydroperoxide in toluene [57], which is suited for most applications in oxidation catalysis.

Oxidation potentials show that hydrogen peroxide and *tert*-butyl hydroperoxide are able to oxidize bromide in aqueous or in polar aprotic solvents to furnish bromine (Table 1). At neutral pH, the two peroxides, however, are surprisingly inert toward bromide and have to be activated by a Brønsted- or a Lewis-acid [58–60]. The activated peroxide converts bromide into hypobromite (BrO^-), which exists in neutral aqueous solution predominantly as undissociated hypobromous acid ($pK_a = 8.7$) [61] (Scheme 3) [58].

2.2. Reactivity of oxovanadium(V) compounds toward nucleophiles and hydrogen peroxide

Vanadate(V) exists in neutral aqueous solution as a mixture of monovanadate (HVO_4^{2-} and $H_2VO_4^-$), divanadate, and tetravanadate. The equilibrium in a concentration range of 1–10 mM of vanadate(V) is in favor of the monomer [70]. Monovanadate reacts in pH-neutral to weakly acidic solution with alcohols [71,72], diols [73], and phenols [74], to furnish esters of orthovanadic acid, that is H_3VO_4 ($pK_a^1 = 3.5$, $pK_a^2 = 7.8$, $pK_a^3 = 12.5$) [75]. If treated with carboxylic acids [76], peptides [77,78], phosphate [79,80], or arsenate [79], monovanadate forms mixed anhydrides. Condensation of monovanadate and hydroxycarboxylic acid [81–83], provides mixed anhydride esters and water. Hydrogen peroxide (pK_a 11.6) and monovanadate ($H_2VO_4^-$) form oxoperoxovanadate(V) (Eq. (4); Scheme 4) [84]. The observed rate constant of this condensation in strongly acidic solution is $k^5 = 5 \times 10^3\ M^{-1}\ s^{-1}$ ($I = 3$ M, 25 °C; Eq. (5)) [85]. From acid and hydrogen peroxide dependence it was concluded that k^5 consists of three terms. One of the terms is directly proportional to proton concentration, the second indirectly, and the third independent from the acid strength. From pressure dependence of these rate constants in the pH-range of 0–1.5 activation parameters for oxoperoxovanadate(V) formation (Eq. (5)) of $\Delta H^{\ddagger} = 21\ kJ\ mol^{-1}$, $\Delta S^{\ddagger} = -69\ J\ mol^{-1}\ K^{-1}$ and $\Delta V^{\ddagger} = 15\ cm^3\ mol^{-1}$ were determined [85]. The equilibrium constant for oxoperoxovanadate(V)-formation in the range between pH 6.7 ($K^4 = 3 \times 10^3\ M^{-1}$; ~20 °C; $I = 1.0$ M) [86] and pH 1 ($K^5 = 3.7 \times 10^4\ M^{-1}$; 25 °C; $I = 0.3$–1.0 M) [87] is only moderately dependent on acid concentration [88].



Scheme 3. Proposed mechanism for peroxide activation and bromide oxidation (R = H or *t*Bu; M = e.g. H or V^V) [42,68,69].

Hydrogen peroxide reacts with monoperoxovanadate(V) to give bisperoxovanadate(V) (Scheme 4). The rate constant at pH 2 (aqueous $HClO_4$) is $k^7 = 3.5 \times 10^3\ M^{-1}\ s^{-1}$ ($I = 3$ M, 25 °C). Activation parameter for this reaction (Eq. (7)) are $\Delta H^{\ddagger} = 40\ kJ\ mol^{-1}$, $\Delta S^{\ddagger} = -42\ J\ mol^{-1}\ K^{-1}$ and $\Delta V^{\ddagger} = 0\ cm^3\ mol^{-1}$ [85]. The equilibrium constant decreases from $K^6 = 2 \times 10^5\ M^{-1}$ (pH 6.7, ~20 °C, $I = 1$ M) [86] to $K^7 = 0.6$ M (pH 1.0, 25 °C, $I = 0.3$) [87], as the solution becomes more acidic.

Even higher concentration and aliquots of hydrogen peroxide provide tris- and tetrakisperoxovanadates from vanadate(V) [89]. The conditions required to prepare tris- and tetrakisperoxo complexes differ from parameters used in oxidation catalysis. Kinetic and thermochemical aspects dealing with tris- and tetrakisperoxovanadate formation therefore are not further considered in this article.

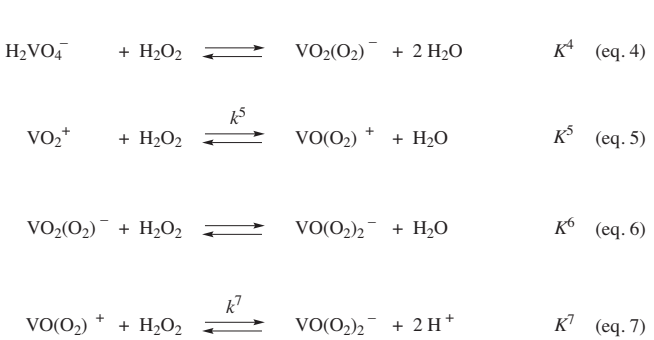
Molecular orbital theory predicts that peroxo coordination between vanadium(V) and oxygen occurs via σ- and π-bonding (Fig. 1). π-Bonding shifts electron density from the pair of non bonding electrons at the peroxide oxygens toward the metal, thus lowering repulsive interactions within the peroxo entity [90]. Transfer of electron density from the ligand to the metal is spectroscopically detectable by a ligand-to-metal-charge-transfer band at 455 nm ($\varepsilon = 278$, in 5×10^{-3} M $HClO_4$), which gives rise to the reddish brown color of oxoperoxovanadate [91]. Bonding of a second peroxo ligand causes the π-bond order to decrease from 1 to 0.5 because p-orbitals from two oxygen atoms now interact with the same vanadium-d-orbital. This model helps to explain the

Table 1
Oxidation potentials of elementary reactions associated with oxidative bromination

$Br^- \rightleftharpoons ½\ Br_2 + e^-$	E^0_1	(eq. 1)
$Br^- + H_2O \rightleftharpoons HOBr + H^+ + 2e^-$	E^0_2	(eq. 2)
$ROOH + 2H^+ + 2e^- \rightleftharpoons ROH + H_2O$	E^0_3	(eq. 3)

Solvent	Eq.	E^0/V[a]
H_2O	(1)	$E^0_1 = 1.09$ [62,63]
CH_3CN	(1)	$E^0_1 = 0.86$ [64]
H_2O[b]	(2)	$E^0_2 = 1.33$ [65]
H_2O[c]	(3) (R = H)	$E^0_3 = 1.35$ [66]
CH_3OH/C_6H_6	(3) (R = *t*Bu)	$E^0_3 = 1.20$ [67]

[a] Versus NHE.
[b] pH 0; 0.76 V at pH 14.
[c] pH 7; 1.76 V at pH 0 and 0.87 V at pH 14 [66].



Scheme 4. Stoichiometry for peroxovanadate (Eqs. 4 and 5) and bisperoxovanadate formation (Eqs. 6 and 7; c_{H_2O} was treated as constant to obtain values and dimensions of K^{4-7} provided in the text) [85–88].

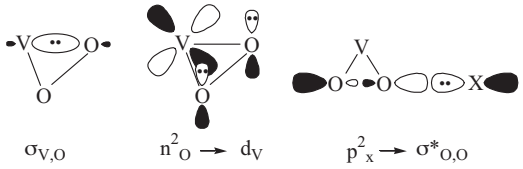


Fig. 1. MO models for peroxo ligand coordination to vanadium via σ- and π-bonding (left and center; n^2_O = pair of non bonding electrons in p-type orbitals at oxygen atoms), and bonding interaction relevant for oxygen atom transfer from peroxovandate(V) to a nucleophile X (X = e.g. Br^-).

hypsochromic shift of the ligand-to-metal-charge-transfer band to λ_{max} = 350 nm (ε = 310) in the pale yellow oxobisperoxovandate(V) ion [91].

Binding to vanadium(V) lowers the energy of the oxygen–oxygen σ^*-orbital of the peroxide, which facilitates oxygenation of a nucleophile (Fig. 1, right) [92,93]. Although molecular orbital theory predicts that the π-bond order between the peroxo oxygen atoms and vanadium in bisperoxovanadates is lower than in monoperoxovanadates, the bisperoxo complex is generally less reactive toward nucleophiles than the monoperoxo form. The origin of this dichotomy is the acid strength of neutral bisperoxovanadate, that is $VO(O_2)_2H$ [pK_a = 0.43] [91]. In neutral solution, the bisperoxo compound exists predominantly as anion and therefore repels an incoming nucleophile. Protonation of a bisperoxovanadate, in turn, enhances bisperoxo complex reactivity, for example, by a factor of 10^4 for sulfoxidation, if referenced toward reactivity of the monoperoxo form [94].

Dipeptides with carboxyl-, hydroxyl-, or amino-functionalized side chains preferentially bind via the deprotonated amide nitrogen to vanadate(V). The carboxylate oxygen is the second best donor in peptides, which, however, binds stronger to vanadate(V) than the N-terminus. No significant affinity seems to exist for vanadate(V)-coordination to the heterocyclic nitrogen atom of *N*-methyl imidazole, glycyl L-histidine, or L-histidyl glycine. Equilibrium constants for peptide binding to vanadate(V) and its peroxo derivatives at pH 6.7 are generally small [95–97].

Physical organic investigations on haloperoxidases [98,99] show that a protein interacts with vanadate(V) in more complex manner than a dipeptide. The constant for monovandate dissociation from the apoenzyme of the bromoperoxidase I from the brown alga *Ascophyllum nodosum* [V_{Br}PO(*An*I), EC 1.11.1.10] is 55 nM. In the crystal (PDB accession code 1QI9), the vanadate(V) co-factor is attached to the distal imidazol nitrogen of the L-histidine486 side chain. This binding site is situated at the bottom of a substrate funnel, which is about 12 Å wide and 8 Å deep. The substrate funnel is located at the end of a four-helix bundle that constitutes an important structural domain of the 120 kDa-homodimer. X-ray diffraction analysis of a hydrogen peroxide-soaked crystal shows that His486 also is the binding site for the monovanadate(V) co-factor in its peroxo form (Fig. 2). In the absence of bromide, the peroxo-loaded-vanadate co-factor shows little to no catalase activity, and seems to bind stronger to the apoenzyme than monovanadate [100,101]. Experimentally, no evidence so far exists that a second hydrogen peroxide molecule enters the monoperoxovanadate coordination sphere of the loaded co-factor [102]. For reasons given above, protein-bound bisperoxovanadate(V) is expected to be a less reactive oxidant for bromide than the monoperoxo form.

Differences in binding affinities of vanadate(V) toward dipeptides and the apoenzyme of V_{Br}PO(*An*I) point to a decisive role of hydrogen bonding between the co-factor, amino acid side chains, and presumably water. On the basis of computational analysis, hydrogen-bonded water molecules at the active site play an essential role for rapid hydrogen peroxide-loading of vanadate, and its activation for bromide oxidation [103]. This water structure would

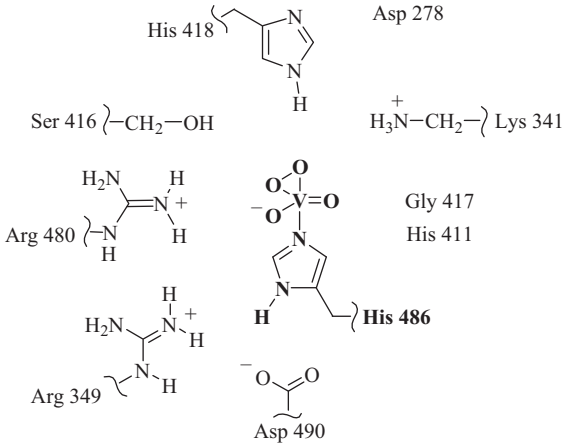


Fig. 2. Proposed structure of the active site in peroxo-loaded V_{Br}PO(*An*I) (adapted from X-ray diffraction data; numbers refer to amino acid sequence of the protein) [98].

collapse, if the apoenzyme was removed from the co-factor, to leave monovandate in homogeneous aqueous solution.

2.3. Alkylperoxy complexes of oxovanadium(V)

Oxovanadium(V) compounds undergo ligand substitution, if treated with alkyl hydroperoxides (Scheme 5) [59]. The equilibrium constant for the reaction between triethyl vanadate and *tert*-butyl hydroperoxide (pK_a 12.3; Scheme 7, Eq. (8)) is $K^8 = 6 \times 10^{-2}$ ($CDCl_3$, −40 °C), and thus significantly smaller than the reference value for monoperoxo complex formation [104]. Substitution of *tert*-butyl peroxy for ethoxy is detectable, for example, via mass spectrometry or vanadium-51 NMR-spectroscopy, but generally not by UV/vis-spectroscopy, because electronic changes associated with the substitution are comparatively small [41,104,108].

Binding to a d^0-transition metal, such as vanadium(V), is a prerequisite for activation and the use of an alkyl hydroperoxide as terminal oxidant in oxidation catalysis [42,60,105,106]. According to molecular orbital theory, coordination between the distal oxygen (O^d) and vanadium occurs via σ- and π-bonding (Fig. 3). In extension to the principles of structural peroxide chemistry, it is expected that VOOR (e.g. R = *t*Bu) preferentially adopts a gauche conformation to minimize lone pair repulsion. In the gauche conformer $n^2_O \rightarrow \sigma^*_{V,O}$ – and $n^2_O \rightarrow \sigma^*_{C,O}$ – interactions reduce Coulomb repulsion within the peroxy entity. The sp^2-type lone pair at the proximal oxygen (O^p), in this model, does not point in a noteworthy manner toward vanadium. A perester-type alkylperoxy binding, which means an end-on coordination, therefore is favored. This interpretation deviates from the crystal structure analysis of oxo[2,6-dipicolinato(−2)](*tert*-butylperoxy)vanadium(V), which so far is the only report in the literature on a solid state structure of an alkylperoxy complex [107]. In the investigated crystal, the *tert*-butyl peroxy ligand is side-on bound. In solution and in the gas phase, however, the end-on mode of binding seems to be the adequate structural model to describe alkyl peroxy binding to vanadium(V) [108].

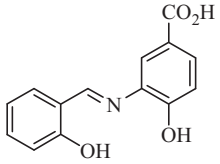
3. Bromoperoxidase catalysis

In the decade that followed the discovery of vanadate(V)-dependent bromoperoxidases (V_{Br}POs), several experiments were performed using enzymes from different organisms, inconsistent enzyme/substrate-ratios, and a variety of buffers to conduct oxidative transformations of nucleophiles. The results showed that bromoamine formation, bromocyclization, indole function-

$VO(OEt)_3 + t\text{BuOOH} \rightleftharpoons VO(OEt)_2(OOt\text{Bu}) + EtOH \quad K_8$ (eq. 8)

$VOL^1(OEt) + t\text{BuOOH} \longrightarrow VOL^1(OOt\text{Bu}) + EtOH$ (eq. 9)

$\delta = -538$ $\delta = -578$

$H_2L^1 =$ 

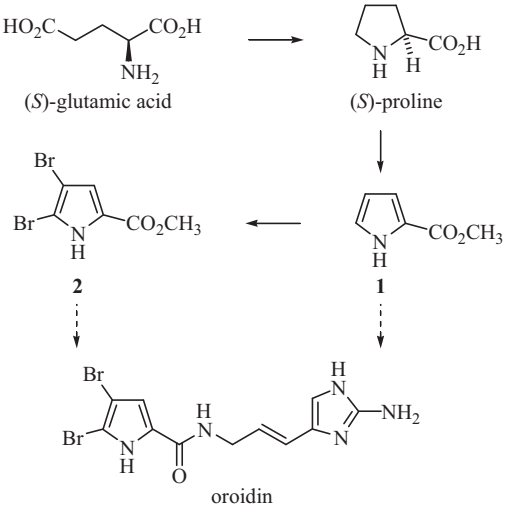
Scheme 5. Oxoperoxyvanadium(V) compound formation from triethyl vanadate (top) [104] and an oxovanadium(V) Schiff-base complex (bottom) [41].

alization, and bromohydrin synthesis are feasible under such conditions [40,109]. None of the reports, however, provided information relevant to organic synthesis, such as yields, mass balances, turnover numbers, catalyst lifetimes in different buffers, or residual activity for repetitive use of bromoperoxidases. Also, no functional group systematic was set up to predict selectivity for natural product transformation via bromoperoxidase-catalyzed oxidation. The aspect of functional group systematic was recently addressed in a study on methyl pyrrole-2-carboxylate bromination (Schemes 6 and 7) [110]. Methyl pyrrole-2-carboxylate (**1**) is a metabolite from the amino acid pathway [111], and gives a diagnostic mixture of bromination products from electrophilic aromatic substitution, to provide insight into reactivity and selectivity of bromoperoxidase chemistry [110,112].

The selected enzyme, that is the $V_{Br}PO(AnI)$, catalyzes oxidation of bromide with hydrogen peroxide in weakly acidic solutions (pH 6.2–6.5) [37]. The turnover rate of this process exceeds the most active nonenzymatic alternatives by four orders of magnitude [39]. The enzyme tolerates organic co-solvents, such as alcohols, 1,4-dioxane, acetonitrile, and temperatures of up to 60 °C, without notably loss of bromoperoxidase activity over time spans that are required to perform synthesis [100,113]. Vanadate-dependent bromoperoxidases from other organisms than *A. nodosum*, for example from fungi or lichens, have been isolated [36,114]. None of theses enzymes, however, shows similarly favorable characteristics for application in organic synthesis as $V_{Br}PO(AnI)$ [25,26,115].

3.1. Bromoperoxidase preparations

In the time between January and April, *A. nodosum* exhibits particularly high levels of $V_{Br}PO(AnI)$ [35,116], which makes isolation of the bromoperoxidase attractive during this season. An established freeze–drying, milling, and liquid–liquid partitioning process provides a mixture of isoenzymes, which must



Scheme 6. Proposed biosynthetic relationship between methyl pyrrole-2-carboxylate (**1**), compounds prepared from sustainable bromination, and natural products [110,111].

be separated via hydrophobic interaction and size exclusion chromatography [116]. Preparations of the $V_{Br}PO(AnI)$ obtained from this procedure exhibit peroxidase activity of up to 693 units U_T mg^{-1} in the triiodide test [117] (pH 6.3). In this test iodide is oxidized into triiodide, as expressed with the index in U_T. One unit (1 U) thereby refers to the amount of enzyme necessary for turning over 1 μmol of substrate per minute. The specific activity, on the other hand, refers to the number of units per mg of enzyme, that is, U_T mg^{-1}. The enzymatic activity in the monochlorodimedone assay [34] was up to 172 U_{MCD} mg^{-1} (pH 6.5). In the MCD-assay, the time dependent decrease of the MCD-absorbance at $\lambda_{max} = 290$ nm

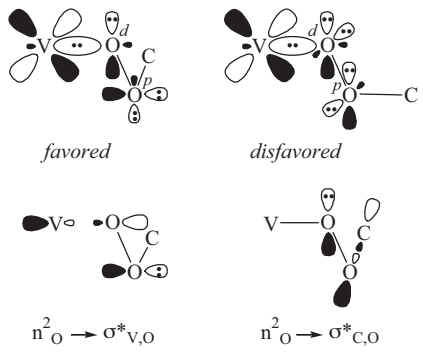
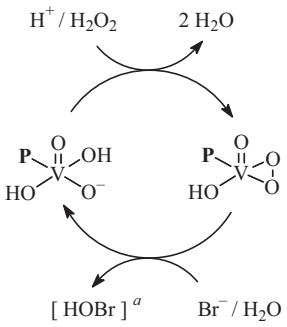
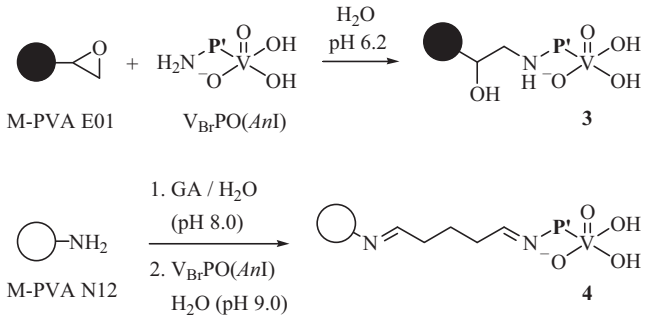


Fig. 3. Molecular orbital model for alkylperoxy binding to vanadium(V) (C refers to a carbon substituent such as *tert*-butyl; n^2_O = p-type lone pair at oxygen).



Scheme 7. Stoichiometry for $V_{Br}PO(AnI)$-catalyzed bromide oxidation (**P** = protein, i.e. apoenzyme; [a] proposed intermediate in an early phase of the reaction; see also Section 5).



Scheme 8. Immobilization of the $V_{Br}PO(AnI)$ on magnetic (M) polyvinyl alcohol (PVA)-coated supports (0.5–1.3 μm polydisperse size distribution for epoxide functionalized beads E01 ○, and 0.7–4.5 μm for amino-functionalized beads ●; GA = glutardialdehyde) [118].

is correlated with the rate of 2-bromo-2-chlorodimedone formation.

The $V_{Br}PO(AnI)$ was immobilized on magnetite particles for collecting the enzyme after substrate turnover from the reaction mixture via magnetic separation with a neodyme/iron bar magnet (Scheme 8, Fig. 4) [118]. The bromoperoxidase retains about 30–40% of its initial activity, upon attachment to the solid phase. Long term measurements show that preparations stored in Tris–HCl buffer (pH 9.0, 4 °C) lose about a third of their bromoperoxidase activity within the first 70 days. For the immobilized $V_{Br}PO(AnI)$, such as preparations **3** and **4**, bromoperoxidase activity beyond this point remains approximately constant [100,118].

3.2. Sustainable bromination in homogeneous and heterogeneous systems

Parameter variation showed that a bromoperoxidase activity of 1.3 U_T is necessary to quantitatively convert 36 μmol of pyrrole **1** into 94% of bromopyrrole **5** within 24 h (Table 2, entry 1). Bromoperoxidase reactivity in homogeneous solution and from immobilized preparations, that is **3** and **4**, are approximately similar (Table 2, entries 2–4). The use of morpholine-4-ethanesulfonic acid (MES)-buffer for synthetic application poses a major improvement, compared to the phosphate buffers used in the early days. For example, the half-life time of bromoperoxidase activity of the enzyme is about 5 h, if stored at 4 °C in phosphate buffer (pH 6.3), which extends to about 9 days in MES-buffer (pH 6.2). On the micromolar scale, the hydrogen peroxide may be added in portions or in a single batch, whereas continuous administration is more effective as the process is scaled-up (vide infra).

An alternative to external administration of the oxidant, is the in situ hydrogen peroxide generation via aerobic oxidation

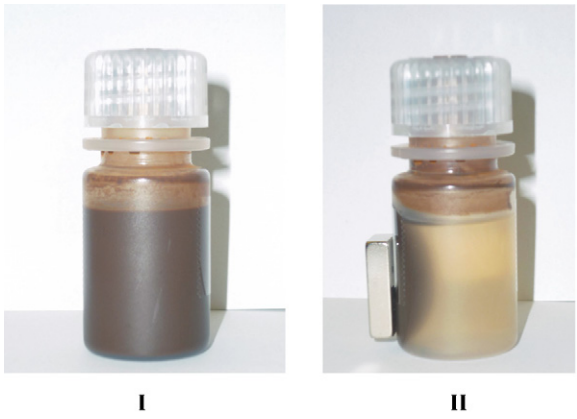
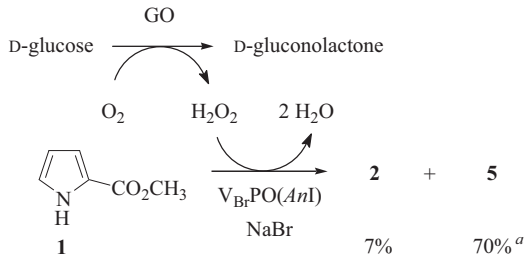


Fig. 4. Illustration of a reaction vessel for $V_{Br}PO(AnI)$-catalyzed oxidation during substrate turnover (**I**) and after magnetic separation of the immobilized enzyme (**II**) (e.g. **3**, **4**) [110].

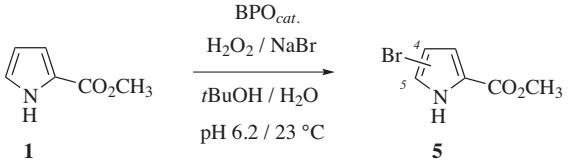


Scheme 9. Combination of two enzymatic processes for pyrrole bromination (GO = D-glucose oxidase; [a] 77/23-mixture of 4/5-isomers of **5**) [118].

of D-glucose, catalyzed by D-glucoseoxidase (GO) [119,120] from *Aspergillus niger* (1.67 U, 147 U mg^{-1}). In order to effectively combine the two enzymatic processes, the pH of the reaction mixture has to be set to 5.8. This value is a compromise between the pH for maximum activity of the $V_{Br}PO(AnI)$ (6.2) and the applied D-glucoseoxidase (5.6). Pyrrole conversion under such conditions is 97%, to furnish 77% of bromopyrroles **2** and **5** (Scheme 9) [118].

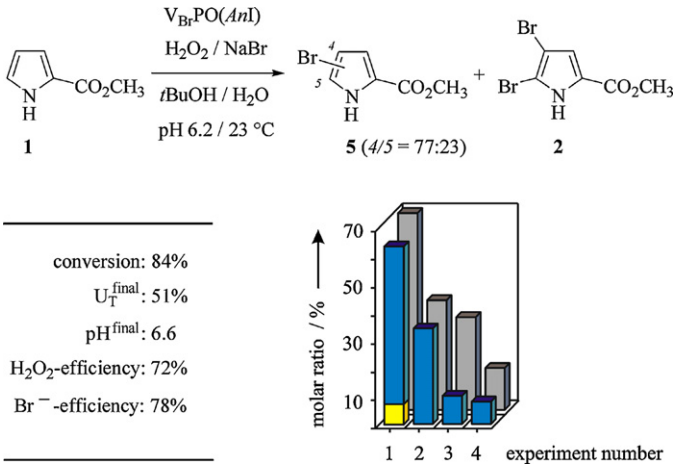
The $V_{Br}PO(AnI)$-activity, that is conserved in oxidative bromination of **1**, may be used for further experiments, leading to a maximum turnover number of 2×10^6 (Scheme 10) [118]. In state-of-the-art oxidation catalysis, it is not only the turnover number of the catalyst that is important but also the efficiency for the use of *all* substrates. To attain maximum efficiency for substrate bromination, sodium bromide (1.3 equivalents), hydrogen peroxide (1.1 equivalents) and MES-buffer must be added continuously to a solution containing 0.75–1.5 mmol of pyrrole **1** and the enzyme at ~20 °C. Regioselectivity of monobromide forma-

Table 2
Reaction parameters for methyl pyrrole-2-carboxylate bromination [118]

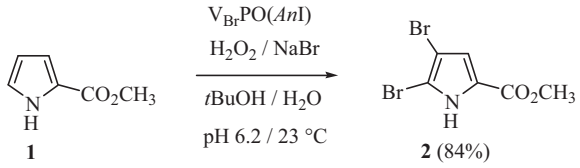


Entry	n_1/μmol	Conv. **1**/%	Time/h	BPO	U_T	U_T^{final} mg^{-1} [a]	**5**/% (4/5)
1	36	Quant.	24	$V_{Br}PO(AnI)$	1.3	117	94 (93/7)
2	36	77	3	$V_{Br}PO(AnI)$	1.3	239	60 (82/18)
3	200	54	3	**3**	6.9	Active	40 (90/10)
4	200	75	3	**4**	6.9	Active	32 (93/8)

[a] Initial enzyme activity 526 U_T^0 mg^{-1} for entries 1–2, 118 ± 12 U_T^0 mg^{-1} for entry 3, and 194 ± 39 U_T^0 mg^{-1} for entry 4; active refers to residual enzyme activity as judged by triiodide tests.



Scheme 10. Efficiency for oxidative methyl pyrrole-2-carboxylate bromination (144 μmol) in sequential reactions [4.96 U_T^0 of $V_{Br}PO(AnI)$; homogeneous MES-buffered solutions; histograms refer to conversion of **1** (gray), and yields of **2** (yellow) and **5** (blue)] [118].



Scheme 11. Synthesis of naturally occurring brominated pyrrole **2** from *A. oroides* [110,121].

tion thereby declines and dibromide **2** is formed as additional product, presumably due to higher reactant concentrations (cf. Scheme 10, experiment 1). The dibromide becomes the major product, if amounts of $V_{Br}PO(AnI)$ (34.6 U_T), sodium bromide, and hydrogen peroxide are doubled. Synthesis of methyl 3,4-dibromopyrrole-2-carboxylate **3a** poses a biomimetic approach to prepare a naturally occuring constituent from the marine sponge *Agelas oroides* (Scheme 11) [121]. The compound shows antiplasmoidal, cytotoxic, and interesting electrophysiologic properties and therefore has attracted attention of medicinal chemists [122,123].

To conserve a higher degree of bromoperoxidase activity for a use in consecutive runs, immobilized $V_{Br}PO(AnI)$ is recommended to be used as oxidation catalyst. For immobilized $V_{Br}PO(AnI)$-preparation **3**, the maximum number of experiments attainable so far is 15 (Fig. 5). The turnover number under such conditions is 1.1×10^6. Although this number is by a factor of two smaller than for the enzyme in homogeneous solution, the use of immobilized bromoperoxidase is by far the more practical method for

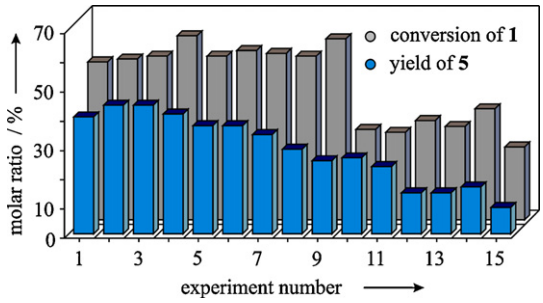


Fig. 5. Histograms for presenting conversion (gray) and yields (blue) for consecutive use of $V_{Br}PO(AnI)$-preparation **3** for oxidative pyrrole bromination (in aqueous *tert*-butanol, pH 6.2, 23 °C) [118].

catalyst recycling. The origin of irreversible bromoperoxidase activity loss in oxidation catalysis is unclear. Attempts to reconstitute bromoperoxidase activity by orthovanadate addition failed.

3.3. Bromination of arenes

Substituted benzenes are models for acetogenins and shikimates, to investigate selectivity of arene bromination in bromoperoxidase-catalyzed oxidation (Table 3). Brominated phenols, for example, were isolated from marine sponges of the genera *Didiscus* or *Dysidea* (Scheme 12, bottom) [124,125].

The substrates chosen for probing reactivity of arene bromination in $V_{Br}PO(AnI)$-catalyzed oxidations differ in turnover efficiency. The yields of products **7**–**8** under conditions that are limited in hydrogen peroxide (1.0 equivalent) and sodium bromide (1.1 equivalents), decrease along the series of substrates aniline (**6a**) > phenol (**6b**) > *O*-methyl thymol (**6f**) > 2-*tert*-butyl phenol (**6c**) > thymol (**6d**) > anisole (**6e**) (Table 3, entries 1–6) [126]. Evidence for sidechain bromination of, for example, isopropyl-substituted benzenes **6d** and **6f** were not apparent from NMR-spectra of associated reaction mixtures [126]. *tert*-Butylbenzene, chlorobenzene, and methyl benzoate were not brominated under such conditions, even as aliquots of hydrogen peroxide and sodium bromide were increased by a factor of three. Triiodide tests showed that $V_{Br}PO(AnI)$-activity in all instances is retained. The lack in reactivity therefore is not caused by enzyme inhibition, either by the substrate or the product(s), but points to insufficient reactivity of the applied arenes.

α-Naphthol **9**, a model for naturally occurring hexaketides, is converted in a bromoperoxidase-catalyzed reaction into a 65/35-mixture of 4/2-isomers of bromonaphthol **10** [127,128]. Substrate, reactant, and buffer were added continuously. Enzymatic activity (17.3 U_T for 0.75 mmol of **9**) was lost at a substrate conversion of about 58%. Attempts to brominate 1-methylnaphthalene under standard conditions led to phase separation without affecting enzyme activity. Bromination of 1-methylnaphthalene, however, did not occur under such conditions.

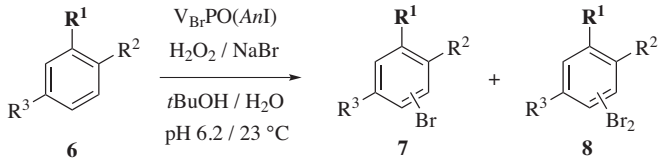
4,6,8-Trimethylazulene (**11**) [129], a compound of similar reactivity in electrophilic aromatic substitution as pyrrole, undergoes 20% conversion (formation of 19% of bromoazulene **12**), if treated with sodium bromide and hydrogen peroxide in aqueous, MES-buffered *tert*-butanol. If the reaction is performed in aqueous acetonitrile containing diethyl ether as additional co-solvent, and dodecyl trimethyl ammonium bromide (DTAB) as phase transfer catalyst, the yield of brominated azulenes **12** and **13** [130] increased to a total value of 71% (Scheme 13, top). The end of substrate conversion correlated with the loss of bromoperoxidase activity. Brominated azulenes are natural products. 1-Bromoguiazulene, for example, is a bluish purple pigment of the deep sea gorgonian *Euplexaura erecta* [131].

To sum up, bromoperoxidase-catalyzed oxidation of bromide in solutions of hydrogen peroxide is the basis for synthesis of bromoarenes from π-nucleophilic aromatics. The method is a sustainable version of the on-site halogenation, and provides insight into selectivity of biomimetic organobromine synthesis.

4. Functional bromoperoxidase mimics

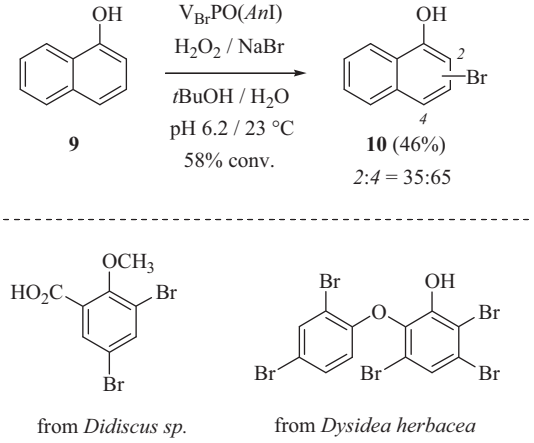
Transition metal complexes, that are able to catalyze the oxidation of bromide, are named functional bromoperoxidase mimics [42,132,133]. The difficulty to use bromoperoxidase mimics in synthesis of complex natural products arises from the fact that one proton is consumed per equivalent of oxidized bromide (Scheme 14). In order to maintain turnover rates for bromide oxidation at a reasonable level, protons must be supplied from an external source. In the first generation of bromoperoxidase mim-

Table 3
Bromination of substituted in $V_{Br}PO(AnI)$-catalyzed oxidations [126]



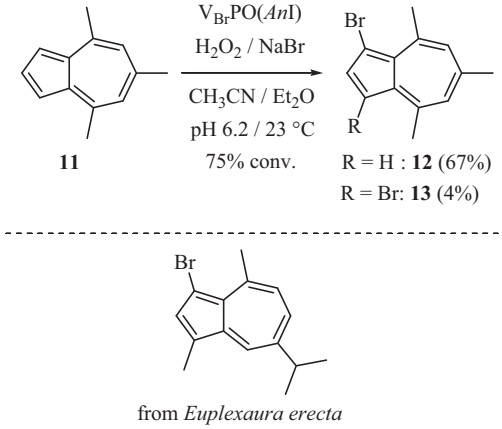
Entry	7	R^1	R^2	R^3	Conv. 6/%[a]	7/% (o/p)[b]	8/% (o,o/o,p)[b]
1	**6a**	NH_2	H	H	93	**7a**: 66 (45/55)	**8a**: 20 (20/80)
2	**6b**	OH	H	H	90[c]	**7b**: 69 (9/91)	**8b**: 1 (<2/98)
3	**6c**	OH	*t*Bu	H	62	**7c**: 33 (30/70)	**8c**: 11 (<2/98)
4	**6d**	OH	*i*Pr	CH_3	46	**7d**: 34 (18/82)	**8d**: 1 (<2/98)
5	**6e**	OCH_3	H	H	22	**7e**: 18 (<2/98)	**8e**: –[e]
6	**6f**	OCH_3	*i*Pr	CH_3	64	**7f**: 53 (<2/98)	**8f**: –[e]

[a] 34.6 U_T for 1.5 mmol of **6**.
[b] Referenced versus R^1; *o* = *ortho*; *p* = *para*.
[c] Additional product: 6% of 2,4,6-tribromophenol.
[e] Not detected.

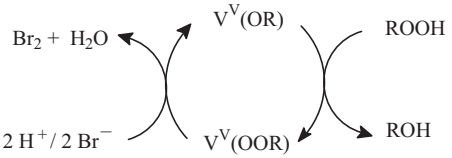


Scheme 12. Bromination of α-naphthol **9** (top) and structure formula of naturally occurring brominated phenol derivatives (bottom; compare also products shown in Table 3) [110,124,125].

ics, this prerequisite was met by running oxidative bromination in strongly acidic aqueous solutions (pH ∼ 1) [68,69]. Such conditions, however, are incompatible with the stability of most organic functional groups and therefore precluded a wider spread use of this method in natural product synthesis. A reexamination of published procedures moreover showed that none of the existing methods



Scheme 13. Bromination of 4,6,8-trimethylazulene **11** (top; 8.65 U_T^0 for 0.375 mmol of **11**) and structure formula of a bluish purple pigment of a deep see gorgonian [110,131].



Scheme 14. Reaction cycle for bromide oxidation using a vanadium(V)-based functional bromoperoxidase mimic [V^V(OR); R = e.g. *t*Bu] as catalyst [42].

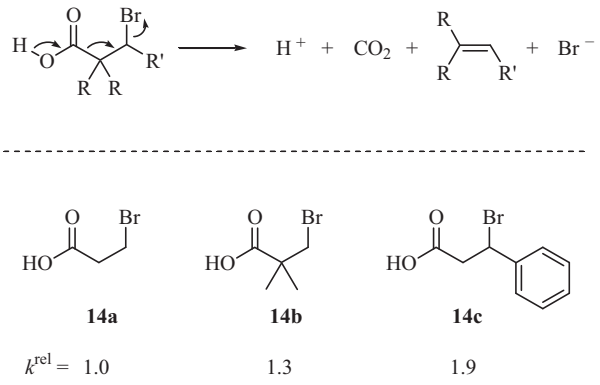
is able to maintain the catalytic cycle for oxidative bromination at neutral pH [26]. Either strong aqueous Brønsted-acids (pH < 2–3) or substantial amounts of vanadium(V) reagents in aqueous solutions were essential to achieve notable substrate conversion for reasons given above. In all instances, the acidity of the reaction mixture drove peroxide activation and thus bromide oxidation. This finding agrees with results from the pioneering study of Maass and Hiebert, who published in 1924 that hydrogen peroxide oxidizes hydrogen bromide in a rapid and exothermic reaction, to give bromine [58]. No external catalyst is needed!

4.1. Masked hydrogen bromide-equivalents

The quest for a proton source that does not notably affect acid strength of an organic solution led to the discovery of the 3-bromopropionic acids, such as **14a**–**c**, as buffer reagents. The compounds are available from renewable resources or low-priced bulk chemicals, and decompose if treated at ambient temperature with catalytic amounts of bromide in solutions of dimethyl carbonate (DMC), propylene carbonate (PC), or ethyl acetate (EtOAc). Fragmentation of 3-bromopropionic acids furnishes a proton, a bromide ion, carbon dioxide, and an alkene [134]. Relative rates of fragmentation correlate with stability of the resulting alkene (Scheme 15).

If *tert*-butyl hydroperoxide is added to a solution of a 3-bromopropionic acid **14** and a catalytic amount of sodium bromide in, for example, propylene carbonate, no bromide oxidation occurs, as probed by addition of 3-*tert*-butylcyclohexene (**15**) to quench possibly formed bromoelectrophiles. Addition of 1 mol% of Schiff–Base complex $VOL^2(OEt)(EtOH)$ [41,135], aminodiol-derived coordination compound $VOL^3(OEt)$, pyridine analogue $VOL^4(OEt)$ [136], $VO(acac)_2$ (Hacac = pentane-2,5-dione), or $VOSO_4{\cdot}4H_2O$ as low-priced alternative (Fig. 6; Table 4, entries 1–5), induces rapid and stereoselective conversion of cycloalkene **15** into dibromocyclohexane **16** (Table 4).

From parameter and reagent variation it was concluded that piperidine complex $VOL^3(OEt)$ in combination with **14b**, *tert*-butyl



Scheme 15. Relative reactivity of 3-bromopropionic acid-fragmentation [134].

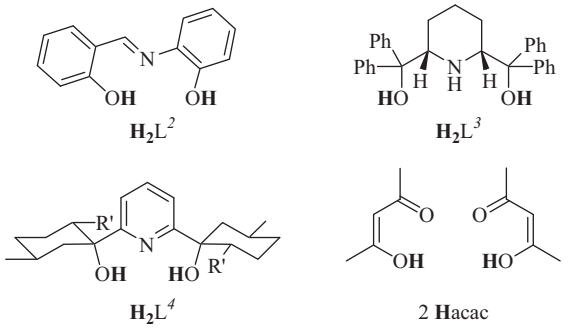


Fig. 6. Structure formulae of auxiliaries used for synthesis of functional bromoperoxidase mimics [cf. Table 4; protons that are released as auxiliaries coordinate to vanadium(V) are printed in bold].

hydroperoxide, and 40 mol% of cyclohexa-1,4-diene (CHD) as co-reductant poses an efficient reagent combination to convert an alkene into a vicinal dibromide. Addition of cyclohexa-1,4-diene accelerates conversion of alkene **15** by a factor of three and improves the yield of dibromide **16** by 12% points to 84%. In the absence of cyclohexa-1,4-diene, additional products appear, such as 3-*tert*-butylcyclohex-5-enone. Isolation of dibromide **16** from propylene carbonate solution is feasible by extraction with cyclohexane and distillation of the hydrocarbon-extract. The cyclohexane extraction leaves a yellow propylene carbonate solution containing the catalyst. To resume oxidative bromination, aliquots of cycloalkene **15**, *tert*-butyl hydroperoxide, and bromopropionic acid **14b**, simply have to be added.

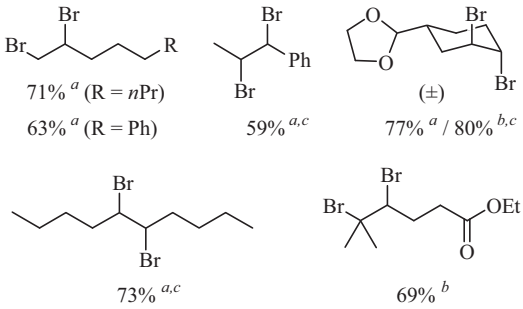


Fig. 7. Products of vicinal dibromination of alkenes from vanadium-catalyzed oxidations (for conditions see text) in propylene carbonate[a] or ethyl acetate[b]; [c]diastereomerically pure [134].

4.2. Dibromination of alkenes

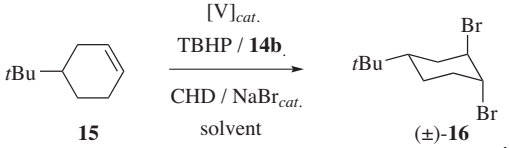
Almost all substitution patterns of alkenes that occur in nature provide dibromides, if treated with the reagent combination of $VOL^3(OEt)$, *tert*-butyl hydroperoxide, and **14b** (Fig. 7). (*E*)- and (*Z*)-configured alkenes are transformed into diastereomerically pure dibromides. pH-measurements of hydrolyzed samples show that no acid accumulates in the course of 3-bromopropionic acid turnover, which allows to brominate acid labile substrates according to this method (Fig. 7). Bromination of polar alkenes provides products that are not quantitatively extractable from solutions of propylene carbonate with cyclohexane. In such instances, ethyl acetate is recommended as solvent for conducting the oxidative bromination [134].

4.3. Bromocyclizations

(*R*)-Linalool (**17a**) provides a 47/53-mixture of brominated tetrahydrofuran **18a** and tetrahydropyran **19a**, if treated with $VOL^3(OEt)$, **14b**, and *tert*-butyl hydroperoxide (Scheme 16, top) [134]. Vicinal bromohydrin ethers **18a** and **19b** are structural related to a number of marine secondary metabolites, such as furoplocamioid A [137] and aplysiapyranoid A [138] (Scheme 16, bottom).

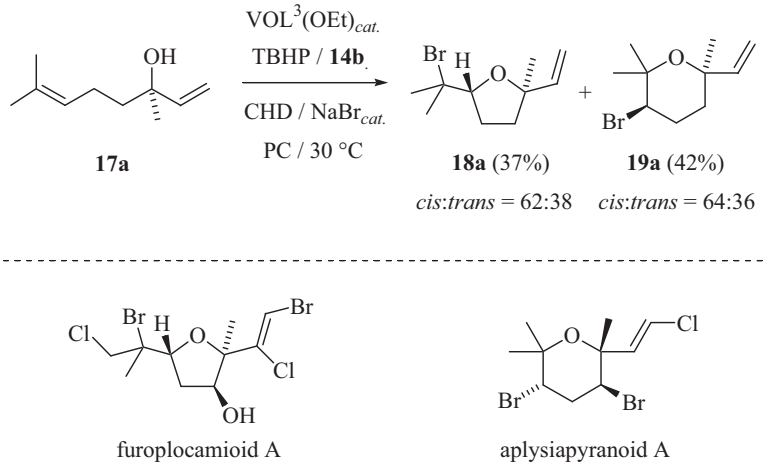
The reagent combination of a 3-bromopropionic acid, *tert*-butyl hydroperoxide and a bromoperoxidase mimic seems to be a quite general system to bromocyclize alkenols, such as **17b–e**, which differ from linalool **17a** in the chemical nature of substituents attached to the π-bond. As the ε-substituent of the alkenol changes from phenyl via methyl to hydrogen, the fraction of 6-endo-cyclized product **19** thereby decreases in a synthetically interesting manner, which is explained below (Table 5, entries 1–5).

Table 4
The use of 2,2-dimethyl-3-bromopropionic acid (**14b**) as in situ hydrogen bromide source in oxidative bromination of cycloalkene **15** (30 °C)[a]



Entry	$[V]_{cat.}$ (mol%)	Additive	MBr	Solvent	(±)-**16**/%
1	$VOL^2(OEt)(EtOH)$	CHD	NaBr	PC	44
2	$VOL^3(OEt)$	CHD	NaBr	PC	**84**
3	$VOL^4(OEt)$	CHD	NaBr	PC	52
4	$VO(acac)_2$	CHD	NaBr	PC	76
5	$VOSO_4{\cdot}4H_2O$	CHD	NaBr	PC	42
6	$VOL^3(OEt)$	None	NaBr	PC	72
7	$VOL^3(OEt)$	CHD	NBu_4Br	DMC	74
8	$VOL^3(OEt)$	CHD	NBu_4Br	EtOAc	83

[a] Conditions: 1.25 equiv. of **14b**, 1.1 equiv. of TBHP, 30 °C, 0.1 equiv. MBr, 1% $[V]_{cat.}$



Scheme 16. Products of linalool bromocyclization (top) and structurally related natural products (bottom) [134,137,138].

The yields in oxidations catalyzed by functional bromoperoxidase mimics compare to results obtained from conventional procedures that, however, applied stoichiometric amounts of *N*-bromosuccinimide or 2,4,4,6-tetrabromocyclohexa-2,5-dienone in acetonitrile, nitromethane, or dichloromethane, to achieve bromocyclization [139–141].

To sum up, alkenes and alkenols afford products of oxidative bromination, if treated at 30 °C with a 3-bromopropionic acid, such as **14a–c**, and *tert*-butyl hydroperoxide in an organic carbonate or ethyl acetate. The reaction is catalytic in vanadium(V) and bromide.

5. Mechanistic considerations

Bromoperoxidase-catalyzed oxidations occur in water and require hydrogen peroxide as oxidant, while oxidations catalyzed by functional mimics, for reasons of selectivity, are preferentially conducted in polar aprotic organic solvents and use *tert*-butyl hydroperoxide as terminal oxidant. Nevertheless, parallels between the methods exist that justify a unified discussion of mechanistic aspects of organic substrate bromination in the following chapters.

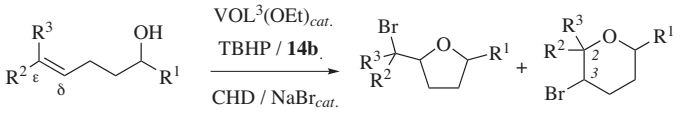
5.1. Equilibria and reactivity in aqueous solution

Hypobromous acid is the primary product of bromoperoxidase-catalyzed oxidation in an aqueous solution of sodium bromide and hydrogen peroxide. Hypobromous acid and bromide react to afford bromine (Eq. (10)), which is transformed by additional bromide to give tribromide as major product (Eq. (11)), and pentabromide (Br_5^-), and bromate (BrO_3^-) as minor products [142–144].

The law of mass action for Eq. (10) transforms on the assumption of infinitesimal substrate concentrations ($I = 0$ M) into $lg(c_{HOBr}/c_{Br_2}) = -7.05 + pH - lg\, c_{Br^-}$. This correlation shows, that the fraction of bromine in an aqueous solution of hypobromous acid increases, as the pH decreases and bromide-concentration rises (Fig. 8).

Selectivity profiles of arene bromination in $V_{Br}PO(AnI)$-catalyzed reactions, and bromine-mediated electrophilic aromatic

Table 5
Synthesis of vicinal bromohydrin ethers from alkenols [134]



Entry	17	R^1	R^2	R^3	CHD	18/% (*cis*:*trans*)	19/% (*cis*:*trans*)
1	**17b**	Ph	H	H	–	**18b**: 51 (29:71)	**19b**: –[a]
2	**17c**	Ph	CH_3	H	CHD	**18c**: 48 (34:66)	**19c**: 21 (>98:2)
3	**17d**	Ph	CH_3	CH_3	CHD	**18d**: 14 (41:59)	**19d**: 68 (96:4)
4	**17e**	H	Ph	H	CHD	**18e**: <5[b]	**19e**: 71(<2:98)[c]

[a] Not detected (^{1}H NMR).
[b] Traces
[c] Refers to relative configuration at C2 and C3.

Table 6
Physical and chemical properties of selected bromination reagents.

Reagent	EA/eV	IP/eV	EN[a]	$BDE_{Br,X}$/kJ mol^{-1}
Br_2	2.51 ± 0.10 [148]	10.52 ± 0.01 [149]	2.96	111.9 ± 0.2 [150]
Br_3^-	–[b]	4.1 [151]	1.97	42–54 [152]
HOBr	1.00 [153]	10.638 ± 0.001 [154]	2.91	202 ± 3 [155]

[a] Electronegativity (EN) of bromination reagents calculated according to Sanderson's principle of group electronegativity [156], using atomic electronegativity values from the Pauling scale [157].
[b] Not available.

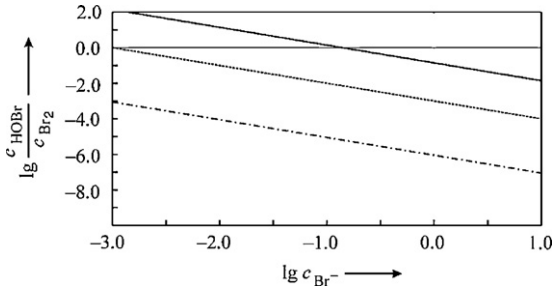


Fig. 8. Double logarithmic plot derived from Eq. (10) for $I = 0$ M at pH 6.2 (– – –), 4.1 (·····), and 1.0 (– · – ·; bottom).

substitutions, are nearly identical [110,130,158]. Molecular bromine is a strong electrophile that rapidly reacts with π-nucleophilic aromatics. Electrophilicity of bromine is reflected by its electron affinity (EA), whereas nucleophilicity of the arene is given by its ionization potential (IP) (Table 6). In frontier molecular orbital (FMO)-theory [159], the electron affinity correlates with the energy of the lowest unoccupied molecular orbital (LUMO) and the ionization potential with the highest occupied molecular orbital (HOMO). For reactions between bromine and nucleophilic arenes, such as phenol (**6b**) (IP = 8.5 eV), anisole (**6e**) (8.4 eV), pyrrole (IP = 8.2 eV), aniline (**6a**) (7.7 eV), and azulene (7.4 eV), favorable $LUMO_{Br_2}$–$HOMO_{arene}$ interactions exist in the transitions states as evident from correlation diagrams [for anisole (**6e**) see Fig. 9]. Selectivity in this model originates from interactions between the electrophile and the site of the largest HOMO-coefficient of the nucleophile (Figs. 9 and 10). If the energy difference between HOMO and HOMO–1 is small, such as for **1** (Fig. 10), two occupied orbitals interact with the electrophile, which makes interpretation of selectivity more difficult.

In terms of reactivity, a limit of IP < 8.8 seems to exist for an arene to undergo electrophilic aromatic substitution in $V_{Br}PO(AnI)$-catalyzed reactions, as concluded from the observation that ethyl benzoate (IP = 9.3 eV), chlorobenzene (9.1 eV), and *tert*-butylbenzene (~8.8 eV) were inert under such conditions. The fact that 1-methylnaphthalene (IP = 8.1 eV for naphthalene) was not brominated, and bromination of 4,6,8-trimethylazulene required a more lipophilic co-solvent in addition to a phase transfer catalyst to notably occur, shows that the role of substrate lipophilicity on reactivity in bromoperoxidase-catalyzed oxidations needs to be addressed in a future study.

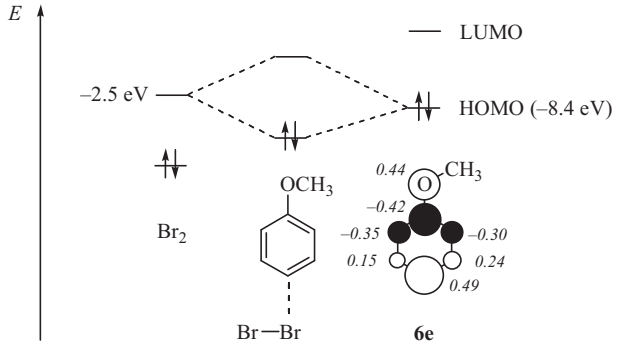


Fig. 9. Correlation diagram displaying most attractive interaction between the LUMO (i.e. σ*) of Br_2 and the HOMO of anisole (**6e**) (HOMO coefficients refer to B3LYP/6-31G-population analysis of B3LYP/6-31 + G**-minimized wavefunction) [159,160].

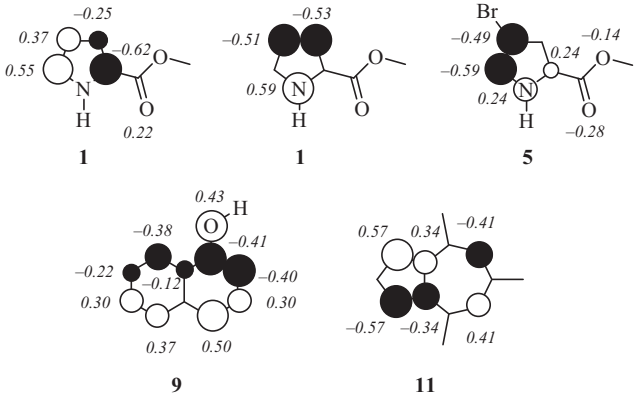


Fig. 10. HOMO coefficients of selected aromatic compounds that undergo electrophilic aromatic substitution in bromoperoxidase-catalyzed oxidations (for **1**: HOMO (top left) and HOMO–1 (top center); coefficients of relative magnitude <0.10 have been omitted for clarity; for theoretical method refer to legend of Fig. 9) [160].

5.2. Selectivity in polar aprotic solvents

In polar aprotic solvents, stereoselectivity of oxidative alkene bromination, and regioselectivity of carbon–oxygen bond formation in bromocyclization, agrees with reactivity of bromine toward π-bonds (Scheme 17).

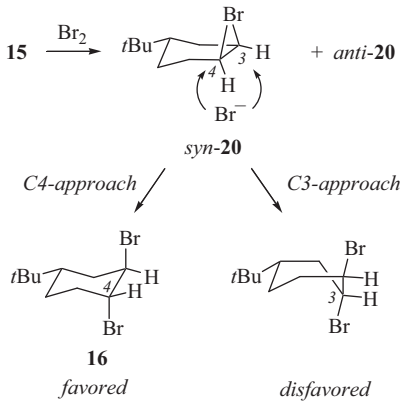
The reaction between bromine and an alkene follows a two-step mechanism. Both steps leave stereochemical fingerprints. In the first step, a cyclic bromonium ion is formed from bromine and the alkene in a rapid irreversible reaction. Selectivity in this step is guided by frontier molecular orbital interactions between bromine and the π-bond of the alkene. In the second step, the cyclic bromonium ion opens in a stereospecific S_N2-type reaction with bromide, via a late and therefore product-like transition state. Charge effects and steric repulsion between substituents are more important for opening of cyclic bromonium ions than for their formation. In this mechanistic picture, cycloalkene **15** provides axially dibromosubstituted cyclohexane **16** because nucleophilic attack at C4 furnishes the product directly in a chair conformation (Scheme 18). Attack of bromide at C3 leads to a high in energy, boat-like conformer, before the structure can relax into a chair conformation, having the two bromosubstituents bound equatorially [161].

In a similar way, selectivity of carbon–bromine- and carbon–oxygen bond formation in bromocyclization reflects a two-steps mechanism that proceeds via a cyclic bromonium ion and nucleophilic opening of this intermediate by backside attack of the hydroxyl oxygen. Carbon–oxygen bond formation from the bromonium ion proceeds via a late transition state. Polar solvent molecules and substituents attached to the π-bond therefore exhibit a pronounced charge stabilizing effect to guide regioselectivity of carbon–oxygen bond formation (Scheme 19). In this mechanistic picture, bromocyclization of (*E*)-configured alkenol **17e** gives 2,3-trans-disubstituted tetrahydropyran **19e** as major product. The phenyl group is able to stabilize positive charge, which weakens (lengthens) the proximal carbon bromine bond, to guide the incoming oxygen nucleophile toward the benzylic car-

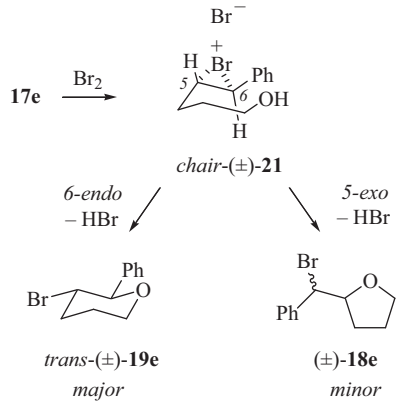
$$HOBr + Br^- + H^+ \rightleftharpoons Br_2 + H_2O \qquad K^{10} \quad \text{(eq. 10)}$$

$$Br_2 + Br^- \rightleftharpoons Br_3^- \qquad K^{11} \quad \text{(eq. 11)}$$

Scheme 17. Equilibria between HOBr, Br_2, and Br_3^- in H_2O [$K^{10} = 1.45 \times 10^8\ M^{-2}$ for H_2O at 20 °C ($I = 0.1$ M, pH 2.6–3.8) [145] or $1.04 \times 10^8\ M^{-2}$ in H_2O at 25 °C (pH 1.5) [146]; $K^{11} = 16.9\ M^{-1}$ in H_2O at 25 °C [147]; $9 \times 10^6\ M^{-1}$ in CH_3CN at 25 °C; $1.5 \times 10^7\ M^{-1}$ in DMC at 25 °C].



Scheme 18. Stereochemical model for explaining selectivity in the synthesis of dibromide **16**.



Scheme 19. Mechanistic model for stereoselective synthesis of tetrahydropyran *trans*-**19e** on the basis of stereoelectronic and polar effects via intermediate bromonium ion chemistry.

bon of the bromonium ion. Regioselectivity of bromocyclizations conducted in organic carbonates favor tetrahydropyran formation more than alternatives using, for example, *N*-bromosuccinimide or 2,4,4,6-tetrabromocyclohexa-2,5-dieneone as bromination reagent in solutions of in dichloromethane or nitromethane [139,141], presumably for reason of such polar effects.

To sum up, thermochemical data imply that tribromide is the major product formed from bromide oxidation with hydrogen peroxide catalyzed by the vanadate(V)-dependent bromoperoxidase from *A. nodosum* and from oxidations using bromoperoxidase mimics in polar aprotic solutions. Tribromide, however, is a nucleophile, as documented by its low group electronegativity of 1.97, compared to bromine (2.96) and hypobromous acid (2.81) (Table 6) [162,163]. Only the electrophilic reagent bromine exhibits adequate reactivity to explain the observed selectivities in oxidative substrate brominations using the enzyme and functional models thereof as catalyst.

6. Concluding remarks

The discovery of the bromoperoxidases completely changed our view on organobromine formation, and challenged scientists to uncover the mechanism of bromide oxidation under physiological conditions. The largest body of mechanistic data so far is available for a vanadate(V)-dependent bromoperoxidase from the brown alga *A. nodosum*. This enzyme combines high affinity toward hydrogen peroxide and sodium bromide with stability toward organic co-solvents. Also, the bromoperoxidase tolerates elevated temperature and significant concentration of organic substrates. To explain these characteristics, we assume that bromide oxidation occurs at the active vandate site at some distance from electrophilic hydrocarbon bromination, which we expect for reasons of reactivity and selectivity to take place in bulk solution. We therefore expect that the bromoperoxidase will find its place as catalyst in synthesis, wherever a sustainable alternative to bromine in water is needed.

The typical reaction medium for the bromoperoxidases is ocean water. Water, however is a nucleophile and therefore able to intercept electrophiles, to change selectivity of, for example, alkenol bromocyclization to vicinal bromohydrin formation. In such instances, water needs to be replaced by a polar aprotic solvent. To achieve bromide oxidation in organic solvents, an alkyl hydroperoxide generally is needed as a more lipophilic oxidant, and peroxide activation is achieved by a functional bromoperoxidase mimic instead of an enzyme. The role of the buffer to deliver protons without changing the acid strength, is taken over in organic solutions by 3-bromopropionic acids (pK_s 4–5). Since the major problems for application of oxidative bromination of acid labile substrates in synthesis were solved in the course of this project, we expect that additional examples that apply this knowledge will be reported in the upcoming years.

By looking at the achievements of bromoperoxidase chemistry, future perspectives, in our opinion, will not only deal with organic synthesis in ocean water, but also with an understanding of the role, the bromoperoxidases play in secondary metabolite synthesis. The structure-reactivity data collected in the past years for vanadate(V)-dependent bromoperoxidases justify the assumption, that electrophilic bromination possibly not always is the final step of a (bio)synthesis [53]. For arenes, a reactivity limit seems to exist, which is guided by the energy of the highest occupied molecular orbital. Methods for crossing this border are not yet discovered. Alkenols furthermore provide racemic vicinal bromohydrins as major components rather than enantiopure products of stereoselective bromocyclization [100]. As the scientific journey to unravel the principles of stereoselective aliphatic carbon–bromine bond formation in natural product chemistry continues, it might be instructive to turn back and see how far chemistry has already come along this way. About three decades ago, the role of organohalogens in science and society was summarized in an excellent textbook as follows.

> Although large numbers of organohalogens are known, very few of them occur naturally. . .. Almost all of the organohalogen compounds in use today are synthetic in origin. Your may wonder why, if nature doesn't choose to make them, man elects to do so. . .. [164]

Nature does these experiments over and over! All it needs is the right hypothesis and the right idea where to look!

Acknowledgements

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References

[1] G.W. Gribble, Acc. Chem. Res. 31 (1998) 141.
[2] K. Ballschmitter, Chemosphere 52 (2003) 313.

[3] G.W. Gribble, Chemosphere 52 (2003) 289.
[4] R. Sander, W.C. Keene, A.A.P. Pszenny, R. Arimoto, G.P. Ayers, E. Baboukas, J.M. Cainey, P.J. Crutzen, R.A. Duce, G. Hönninger, B.J. Huebert, W. Maenhaut, N. Mihalopoulos, V.C. Turekian, R. Van Dingensen, Atmos. Chem. Phys. 3 (2003) 1301.
[5] H. Herrmann, Z. Majdik, B. Ervens, D. Weise, Chemosphere 52 (2003) 485.
[6] R.A. Rasmussen, M.A.K. Khalil, R.W. Dalluge, S.A. Penkett, B. Jones, Science 215 (1982) 655.
[7] S.L. Neidleman, J. Geigert, Biohalogenation, Principles, Basic Roles and Application, Ellis Horwood, Chichester, 1986.
[8] A. Butler, M. Sandy, Nature 460 (2009) 848.
[9] F.H. Vaillancourt, E. Yeh, D.A. Vosburg, S. Garneau-Tsodikova, C.T. Walsh, Chem. Rev. 106 (2006) 3364.
[10] R. Frim, S.D. Ukeles, in: J.E. Kogel, N.C. Trived, J.M. Baker (Eds.), Industrial Minerals & Rocks, 7. ed, Society for Mining, Metallurgy, and Exploration, Littleton, Colorado, 2006, pp. 285–294.
[11] H.H. Landolt, Ber. dtsch. Chem. Ges. 23 (1890) 905.
[12] E.M. Weeks, J. Chem. Educ. 9 (1932) 1915.
[13] A.J. Balard, Ann. Chim. Phys. 32 (1826) 337.
[14] J. Wisniak, Ind. J. Chem. Technol. 9 (2002) 263.
[15] D.L. Turner, J. Food Sci. 37 (1972) 791.
[16] D.A. Laskowski, Rev. Environ. Contam. Toxicol. 174 (2002) 49.
[17] G.H. Wanière, in: S. Patai (Ed.), The Chemistry of Functional Groups – The Chemistry of the Carbon-Halogen Bond, Wiley, Chichester, 1973, pp. 1–47.
[18] J. Green, J. Fire Sci. 14 (1996) 426.
[19] G.W. Gribble, Chem. Soc. Rev. 28 (1999) 335.
[20] P. Friedländer, Ber. dtsch. chem. Ges. 42 (1909) 765.
[21] S. Doonan, in: S. Patai (Ed.), The Chemistry of Functional Groups – The Chemistry of the Carbon-Halogen Bond, Wiley, Chichester, 1973, pp. 865–915.
[22] S.J. Decanio, C.S. Norman, Contemp. Econ. Policy 23 (2005) 376.
[23] J. Kaspersma, C. Doumena, S. Munrob, A.-M. Prinsa, Polym. Degrad. Stab. 77 (2002) 325.
[24] P.B.D. De la Mare, B.E. Swedlund, in: S. Patai (Ed.), The Chemistry of Functional Groups – The Chemistry of the Carbon-Halogen Bond, Wiley, Chichester, 1973, pp. 407–548.
[25] A. Podgoršek, M. Zupan, J. Iskra, Angew. Chem. Int. Ed. 48 (2009) 8424.
[26] G. Rothenberg, J.H. Clark, Green Chem. 2 (2000) 248.
[27] I. Steinhorn, Limnol. Oceanogr. 30 (1985) 451.
[28] I. Gertman, A. Hecht, J. Mar. Sys. 35 (2002) 169.
[29] C.W. Jones, Application of Hydrogen Peroxide and Derivatives, RSC Clean Technology Monographs, Cambridge, 1999, pp. 156–162.
[30] S.M. Jasinski, U.S. Geological Survey Minerals Yearbook – 2006, Bromine, (2007) pp. 14.1–14.6.
[31] J.H. Clark, F.E. Deswarte, in: J.C. Clark, F. Deswarte (Eds.), Introduction to Chemicals from Biomass, Wiley, 2008, pp. 1–20.
[32] H. Raistrick, G. Smith, Biochem. J. 30 (1936) 1315.
[33] M.C.R. Franssen, H.C. van der Plas, Adv. Appl. Microbiol. 37 (1992) 41.
[34] L.P. Hager, D.R. Morris, F.S. Brown, H. Everwein, J. Biol. Chem. 241 (1966) 1769.
[35] H. Vilter, Phytochemistry 23 (1984) 1387.
[36] H. Vilter, in: H. Sigel, A. Sigel (Eds.), Vanadium and its Role in Life, vol. 31, Dekker, New York, 1995, pp. 325–362.
[37] A. Butler, J.V. Walker, Chem. Rev. 93 (1993) 1937.
[38] S.L. Neidleman, J. Geigert, Trends Biotechnol. 1 (1983) 21.
[39] G.E. Meister, A. Butler, Inorg. Chem. 33 (1994) 3269.
[40] A. Butler, J.N. Carter-Franklin, Nat. Prod. Rep. 21 (2004) 180.
[41] M. Greb, J. Hartung, F. Köhler, K. Špehar, R. Kluge, R. Csuk, Eur. J. Org. Chem. (2004) 3799.
[42] J. Hartung, Pure Appl. Chem. 77 (2005) 1559.
[43] J.H. Espenson, O. Pestovsky, P. Huston, S. Staudt, J. Am. Chem. Soc. 116 (1994) 2869.
[44] M.S. Reynolds, S.J. Morandi, J.W. Raebiger, S.P. Melican, S.P.E. Smith, Inorg. Chem. 33 (1994) 4977.
[45] M.S. Reynolds, K.J. Babinski, M.C. Bouteneff, J.L. Brown, R.E. Campbell, M.A. Cowan, M.R. Durwin, T. Foss, P. O'Brien, H.R. Penn, Inorg. Chim. Acta 263 (1997) 225.
[46] D. Schuch, P. Fries, M. Dönges, J. Hartung, J. Am. Chem. Soc. 131 (2009) 12918.
[47] E.S. Huyser, in: S. Patai (Ed.), The Chemistry of Functional Groups – The Chemistry of the Carbon-Halogen Bond, Wiley, Chichester, 1973, pp. 549–607.
[48] J. Hartung, in: E. Schaumann (Ed.), Science of Syntheses, vol. 35, Thieme, Stuttgart, 2006, pp. 287–300.
[49] N. Kreutzkamp, H. Meerwein, R. Stroh, in: E. Müller (Ed.), Houben-Weyl, Methoden der Organischen Chemie, vol. V/4, George Thieme Verlag, Stuttgart, 1960, pp. 679–776.
[50] D. Rehder, Bioinorganic Vanadium Chemistry, Wiley, Chichester, 2008, pp. 105–128.
[51] A. Butler, A.H. Baldwin, Struct. Bond. 89 (1997) 109.
[52] R.M. Moore, Handbook of Enivronmental Chemistry, vol. 3, Part P, Springer, Heidelberg, 2003, pp. 85–101.
[53] J.-M. Kornprobst, Enzyclopedia of Marine Natural Products, vols. 1–3, Wiley–VCH, Weinheim, 2010.
[54] J.W. Blunt, B.R. Copp, W.-P. Hu, M.H.G. Munro, P.T. Northcote, M.R. Prinsep, Nat. Prod. Rep. 26 (2009) 170.
[55] W.H. Richardson, V.F. Hodge, J. Org. Chem. 35 (1970) 4012.
[56] F. Beer, G. Düsing, H. Pistor, in: W. Weigert (Ed.), Wasserstoffperoxid und seine Derivate – Chemie und Anwendung (translates into: hydrogen peroxide and its derivatives – chemistry and application), Hüthig, Heidelberg, 1998, pp. 23–35.
[57] K.B. Sharpless, T.R. Verhoeven, Aldrichim. Acta 12 (1979) 63.
[58] O. Maass, P.G. Hiebert, J. Am. Chem. Soc. 46 (1924) 290.
[59] J. Hartung, M. Greb, J. Organomet. Chem. 661 (2002) 67.
[60] R.A. Sheldon, in: R. Ugo (Ed.), Aspects of Homogeneous Catalysis, vol. 4, Reidel Publishing, Dordrecht, 1981, pp. 3–69.
[61] E.A. Shilov, J. Am. Chem. Soc. 60 (1938) 490.
[62] T. Michalowski, J. Chem. Educ. 71 (1994) 560.
[63] L.G. Lavrenova, T.V. Zegzhda, V.M. Shul'man, Elektrokhimiya 7 (1971) 83.
[64] B. Jakuszewski, S. Taniewska-Osinka, Acta Chim. Soc. Sci. Lodz. 4 (1959) 17.
[65] R. Holze, in: M.D. Lechner (Ed.), Landolt-Börnstein, New Series, IV, Vol. 9A. Physical Chemistry, Electric Properties, Electrode Potentials, Springer, Heidelberg, 2009, p. 41.
[66] T. Sawyer, Oxygen Chemistry, Oxford University Press, New York, N.Y., 1991, p. 25.
[67] C.O. Willits, C. Ricciuti, H.B. Knight, D. Swern, Anal. Chem. 24 (1952) 785.
[68] O. Bortolini, M. Carraro, V. Conte, S. Moro, Eur. J. Inorg. Chem. (2003) 42–46.
[69] G.J. Colpas, B.J. Hamstra, J.W. Kampf, V.L. Pecoraro, J. Am. Chem. Soc. 118 (1996) 3469.
[70] A.S. Tracey, J.S. Jaswal, S.J. Angus-Dunne, Inorg. Chem. 34 (1995) 5680.
[71] A.S. Tracey, B. Galeffi, S. Mahjour, Can. J. Chem. 66 (1988) 2294.
[72] M.J. Gresser, A.S. Tracey, J. Am. Chem. Soc. 107 (1985) 4215.
[73] A.S. Tracey, M.J. Gresser, Inorg. Chem. 27 (1988) 2695.
[74] B. Galeffi, A.S. Tracey, Can. J. Chem. 66 (1988) 2565.
[75] G.R. Willsky, in: D.N. Chasteen (Ed.), Vanadium in Biological Systems, Physiology and Biochemistry, Kluwer, Dordrecht, 1990, p. 4.
[76] A.S. Tracey, H. Li, M.J. Gresser, Inorg. Chem. 29 (1990) 2267.
[77] A.S. Tracey, J.S. Jaswal, F. Nxumalo, S.J. Angus-Dunne, Can. J. Chem. 73 (1995) 489.
[78] J.S. Jaswal, A.S. Tracey, Can. J. Chem. 69 (1991) 1600.
[79] M.J. Gresser, A.S. Tracey, K.M. Parkinson, J. Am. Chem. Soc. 108 (1986) 6229.
[80] A.S. Tracey, M.J. Gresser, B. Galeffi, Inorg. Chem. 27 (1988) 157.
[81] A.S. Tracey, M.J. Gresser, K.M. Parkinson, Inorg. Chem. 26 (1987) 629.
[82] S. Hati, R.J. Batchelor, F.W.B. Einstein, A.S. Tracey, Inorg. Chem. 40 (2001) 6258.
[83] B. Galeffi, A.S. Tracey, Inorg. Chem. 28 (1989) 1726.
[84] A. Butler, M.J. Clague, G.E. Meister, Chem. Rev. 94 (1994) 625.
[85] S. Funahashi, K. Ishihara, M. Inamo, M. Tanaka, Inorg. Chim. Acta 157 (1989) 65.
[86] J.S. Jaswal, A.S. Tracey, Inorg. Chem. 30 (1991) 3718.
[87] F. Secco, Inorg. Chem. 19 (1980) 2722.
[88] M. Orhanović, R.G. Wilkins, J. Am. Chem. Soc. 89 (1967) 278.
[89] T.-J. Won, C.L. Barnes, E.O. Schlemper, R.C. Thompson, Inorg. Chem. 34 (1995) 4499.
[90] J. Hartung, I. Svoboda, in: Z. Rappoport (Ed.), The Chemistry of Functional Groups – The Chemistry of Peroxides, Wiley, Chichester, 2006, pp. 93–144.
[91] V. Conte, F. Di Furia, S. Moro, J. Mol. Catal. 94 (1994) 323.
[92] R.A. Sheldon, in: S. Patai (Ed.), The Chemistry of Functional Groups, Peroxides, Wiley, Chichester, 1983, pp. 162–199.
[93] T. Hirao, Chem. Rev. 97 (1997) 2707.
[94] A.F. Ghiron, R.C. Thompson, Inorg. Chem. 29 (1990) 4457.
[95] A.S. Tracey, J.S. Jaswal, Inorg. Chem. 32 (1993) 4235.
[96] J.S. Jaswal, A.S. Tracey, J. Am. Chem. Soc. 115 (1993) 5600.
[97] A.S. Tracey, J.S. Jaswal, J. Am. Chem. Soc. 114 (1992) 3835.
[98] A. Messerschmidt, R. Wever, Proc. Natl. Acad. Sci. USA 93 (1996) 392.
[99] M. Weyand, H.J. Hecht, M. Kieß, M.F. Liaud, H. Vilter, D. Schomburg, J. Mol. Biol. 293 (1999) 595.
[100] J. Hartung, Y. Dumont, M. Greb, D. Hach, F. Köhler, H. Schulz, M. Časný, D. Rehder, H. Vilter, Pure Appl. Chem. 81 (2009) 1251.
[101] H.S. Soedjak, A. Butler, Biochim. Biophys. Acta 1079 (1991) 1.
[102] D. Rehder, M. Časný, R. Grosse, Magn. Res. Chem. 42 (2004) 745.
[103] D. Geibig, R. Wilcken, M. Bangesh, W. Plass, in: G. Münster, D. Wolf, M. Kremer (Eds.), NIC Series 39, 2008, p. 71.
[104] F. Di Furia, G. Modena, R. Curci, J. Mol. Catal. 14 (1982) 219.
[105] E. Wojaczyńska, J. Wojaczyński, Chem. Rev. 110 (2010) 4303.
[106] F. Baumberger, A. Vasella, Helv. Chim. Acta 66 (1983) 2210.
[107] H. Mimoun, P. Chaumette, M. Mignard, L. Saussine, J. Fischer, R. Weiss, Nouv. J. Chim. 7 (1983) 467.
[108] C. Wikete, P. Wu, G. Zampella, L. De Gioia, G. Licini, D. Rehder, Inorg. Chem. 46 (2007) 196.
[109] V.M. Dembitsky, Tetrahedron 39 (2003) 4701.
[110] D. Wischang, O. Brücher, J. Hartung, Proceedings of the 7th Symposium on the Chemistry and Biological Chemistry of Vanadium, Toyama, Japan, 6th–9th October, 2010.
[111] C.T. Walsh, S. Garneau-Tsodikova, A.R. Howard-Jones, Nat. Prod. Rep. 23 (2006) 517.
[112] C. Schroif-Gregoire, N. Travert, A. Zaparucha, A. Al-Mourabit, Org. Lett. 8 (2006) 2961.
[113] E. de Boer, H. Plat, R. Wever, in: C. Laane, J. Tramper, M.D. Lilly (Eds.), Biocatalysis in Organic Media, Elsevier, Amsterdam, 1987, pp. 317–322.
[114] E.E. Coupe, M.G. Smyth, A. Fosberry, R.M. Hall, J.A. Littlechild, Protein Expr. Purif. 52 (2007) 265.
[115] For guidelines of sustainable synthesis see F.M. Kerton, in: J. Clark, F. Deswarte (Eds.), Introduction to Chemicals from Biomass, Wiley, 2008, Ch. 3, pp. 47–76.

[116] J. Hartung, O. Brücher, D. Hach, H. Schulz, H. Vilter, G. Ruick, Phytochemistry 69 (2008) 2826.
[117] F. Björkstén, Eur. J. Biochem. 5 (1968) 133.
[118] D. Wischang, J. Hartung, T. Hahn, R. Ulber, T. Stumpf, C. Fecher-Trost, Green Chem. 13 (2011) 102.
[119] D. Keilin, E.F. Hartree, Biochem. J. 50 (1952) 331.
[120] M. Hartmann, C. Streb, J. Porous Mater. 13 (2006) 347.
[121] S. Forenza, L. Minale, R. Riccio, E. Fattorusso, J. Chem. Soc. Chem. Commun. (1971) 1129.
[122] G.M. König, A.D. Wright, A. Linden, Planta Med. 64 (1998) 443.
[123] U. Bickmeyer, C. Drechsler, M. Köck, M. Assmann, Toxicon 44 (2004) 45.
[124] N.K. Utkina, S.A. Fedoreyev, S.G. Ilyin, M. Yu, Antipin. Russ. Chem. Bull. 47 (1998) 2292.
[125] B.F. Bowden, L. Towerzey, P.C. Junk, Aust. J. Chem. 53 (2000) 299.
[126] D. Wischang, J. Hartung, manuscript in preparation.
[127] V. Upender, D.J. Pollart, J. Liu, P.D. Hobbs, C. Olsen, W.-R. Chao, B. Bowden, J.L. Crase, D.W. Thomas, A. Pandey, J.A. Lawson, M.I. Dawson, J. Het. Chem. 33 (1996) 1371.
[128] W.J. Militzer, J. Am. Chem. Soc. 60 (1938) 256.
[129] K. Hafner, H. Kaiser, Org. Synth. 44 (1964) 94.
[130] K. Hafner, H. Patzelt, H. Kaiser, Liebigs Ann. Chem. 656 (1962) 24.
[131] M.K.W. Li, P.J. Scheuer, Tetrahedron Lett. 25 (1984) 587.
[132] V. Conte, B. Floris, Inorg. Chim. Acta 363 (2010) 1935.
[133] K. Kikushima, T. Moriuchi, T. Hirao, Tetrahedron 66 (2010) 6906.
[134] O. Brücher, J. Hartung, Proceedings of the 7th Symposium on the Chemistry and Biological Chemistry of Vanadium, Toyama, Japan, 6th–9th October, 2010.
[135] H. Mimoun, M. Mignard, P. Brechot, L. Saussine, J. Am. Chem. Soc. 108 (1986) 3711.
[136] S. Bellemin-Laponnaz, K.S. Coleman, P. Dierkes, J.P. Masson, J.A. Osborn, Eur. J. Inorg. Chem. 7 (2000) 1645.
[137] J. Darias, J. Rovirosa, A. San Martin, A.R. Diaz, E. Dorta, M. Cueto, J. Nat. Prod. 64 (2001) 1383.
[138] T. Kusumi, H. Uchida, Y. Inouye, M. Ishitsuka, H. Yamamoto, H. Kakisawa, J. Org. Chem. 52 (1987) 4597.
[139] J.G. Urones, D. Díez, I.S. Marcos, P. Basabe, A.M. Lithgow, R.F. Moro, N.M. Garrido, R. Escarcena, Tetrahedron 51 (1995) 3691.
[140] J.-C. Harmange, B. Figadère, Tetrahedron: Asymm. 4 (1993) 1711.
[141] T. Gottwald, M. Greb, J. Hartung, Synlett (2004) 65.
[142] G. Jones, S. Baeckström, J. Am. Chem. Soc. 56 (1934) 1517.
[143] H.A. Young, J. Am. Chem. Soc. 72 (1950) 3310.
[144] C.M. Kelley, H.V. Tartar, J. Am. Chem. Soc. 78 (1956) 5752.
[145] M. Eigen, K. Kustin, J. Am. Chem. Soc. 84 (1962) 1355.
[146] J.M. Pink, Can. J. Chem. 48 (1970) 1169.
[147] D.B. Scaife, H.J.V. Tyrrell, J. Chem. Soc. 80 (1958) 386.
[148] T.M. Miller, in: D.R. Lide (Ed.), Handbook of Chemistry and Physics, 78th ed., CRC Press, Boca Raton, 1997, pp. 10–189.
[149] S.G. Lias, in: D.R. Lide (Ed.), Handbook of Chemistry and Physics, 78th ed., CRC Press, Boca Raton, 1997, pp. 10–218.
[150] J.A. Kerr, Chem. Rev. 66 (1966) 465.
[151] G.L. Gutsev, J. Struct. Chem. 30 (1989) 733.
[152] W. Gabes, R. Elst, J. Mol. Struct. 21 (1974) 1.
[153] H. Keller-Rudek, D. Koschel, P. Merlet, U. Ohms-Bredemann, J. Wagner, A. Wietelmann, in: R. Haubold, J.V. Jonanne, H. Keller-Rudek, D. Koschel, P. Merlet, J. Wagner (Eds.), Gmelin Handbook of Inorganic and Organometallic Chemistry, 8th ed., Springer–Verlag, Heidelberg, 1992, Suppl. 2.
[154] B. Ruscic, J. Berkowitz, J. Chem. Phys. 101 (1994) 7795.
[155] M.N. Glukhovtsev, A. Pross, L. Radom, J. Phys. Chem. 100 (1996) 3498.
[156] R.T. Sanderson, J. Chem. Educ. 65 (1988) 112.
[157] S.G. Bratsch, J. Chem. Educ. 62 (1985) 101.
[158] G.A. Olah, Acc. Chem. Res. 4 (1971) 240.
[159] I. Fleming, Grenzorbitale und Reaktionen organischer Verbindungen, VCH, Weinheim, 1979, pp. 56–78.
[160] M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, J.A. Montgomery, Jr., T. Vreven, K.N. Kudin, J.C. Burant, J.M. Millam, S.S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G.A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J.E. Knox, H.P. Hratchian, J.B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R.E. Stratmann, O. Yazyev, A.J. Austin, R. Cammi, C. Pomelli, J.W. Ochterski, P.Y. Ayala, K. Morokuma, G.A. Voth, P. Salvador, J.J. Dannenberg, V.G. Zakrzewski, S. Dapprich, A.D. Daniels, M.C. Strain, O. Farkas, D.K. Malick, A.D. Rabuck, K. Raghavachari, J.B. Foresman, J.V. Ortiz, Q. Cui, A.G. Baboul, S. Clifford, J. Cioslowski, B.B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R.L. Martin, D.J. Fox, T. Keith, M.A. Al-Laham, C.Y. Peng, A. Nanayakkara, M. Challacombe, P.M.W. Gill, B. Johnson, W. Chen, M.W. Wong, C. Gonzalez, J.A. Pople, Gaussian 03, Revision E.01, Gaussian, Inc., Wallingford CT, 2004.
[161] G.W. Klumpp, Reaktivität in der Organischen Chemie, vol. 1, Thieme, Stuttgart, 1977, pp. 22–25.
[162] L. Huai, W. Qingwei, L. Lixin, J. Chem. Educ. 69 (1992) 783.
[163] G.A. Olah, B.G. Balaram Gupta, R. Malhotra, S.C. Narang, J. Org. Chem. 45 (1980) 1638.
[164] J.D. Roberts, M.C. Caserio, in: Basic Principles of Organic Chemistry, 2nd ed., W.A. Benjamin Inc., Menlo Park, 1977, pp. 535–536.