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Parameters for bromination of pyrroles in bromoperoxidase-catalyzed oxidations

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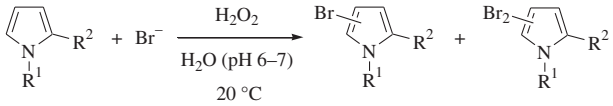
ABSTRACT

Ester-, cyano-, and carboxamide-substituted 1*H*-pyrroles undergo electrophilic aromatic bromination, if treated with hydrogen peroxide and sodium bromide at pH 6.2 and 20 °C. Oxidation of bromide under such conditions is catalyzed by a vanadate(V)-dependent bromoperoxidase, in a substrate/enzyme ratio of 32–63 μmol %. To obtain maximum yields of bromopyrroles (up to 91%) by spending least amount of substrates and catalyst, hydrogen peroxide and sodium bromide have to be added continuously to the enzyme and the 2-acceptor-substituted pyrrole (1.5 mmol) in a solution of morpholine-4-ethanesulfonic acid buffer. This technique was applied to prepare two marine natural products under biomimetic conditions, that is, methyl 4,5-dibromopyrrole-2-carboxylate (from *Agelas oroides*) and 4,5-dibromopyrrole-2-carboxamide (from *Acanthella carteri*).

1. Introduction

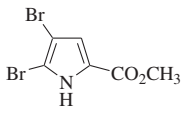
Brominated pyrroles are key building blocks for synthesis of secondary metabolites from the marine environment (Scheme 1).[1] In organic synthesis, bromopyrroles are prepared via electrophilic aromatic substitution of pyrroles, using stoichiometric amounts of *N*-bromosuccinimide, 2,4,4,6-tetrabromocyclohexa-2,5-dieneone, or bromine, to mention the most important bromoelectrophiles.[2–4] From an environmental point of view, ocean water, which is millimolar in bromide is a more foreward-looking resource of the halide to start synthesis of organobromines than the bromoelectrophiles outlined above.[5,6] To bromofunctionalize pyrroles with bromide, one the nucleophilic reactants needs to be converted into an electrophile. For reasons of selectivity, it is the bromide which is oxidized, preferentially with a solution of aqueous hydrogen peroxide. Hydrogen peroxide is a low-priced and safe to handle chemical, which leaves water as the only by-product. In aqueous solution products of peroxide-mediated bromide oxidation transform into an equilibrium mixture of hypobromous acid, bromine, and tribromide, which has notable bromination ability.[7]

The rate of bromide oxidation with hydrogen peroxide under physiological conditions is small.[10] To achieve notable bromide turnover nature uses bromoperoxidases,[11–14] such as the vanadate(V)-dependent bromoperoxidase I from the brown alga *Ascophyllum nodosum* [V_{Br}PO(*An*I), EC 1.11.1.10][15] to catalyze oxidation of bromide at ambient temperature and neutral pH. The bromoperoxidase from *A. nodosum* is a chemically robust oxidation catalyst, which retains its activity if stored in alcohols or heated to ~70 °C.[16,17] Bromoperoxidases from other organisms, such as fungi or lichens, were isolated and screened for reactivity. None of theses

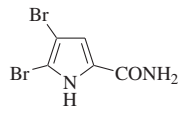


1 **2** **3**

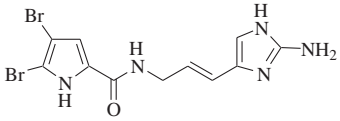
1–3	R^1	R^2
a	H	CO_2CH_3
b	H	$CO_2C_3H_5$
c	H	$CO_2C_5H_{11}$
d	H	$CONH_2$
e	H	CN
f	C_2H_5	CO_2CH_3



3a
Agelas oroides



3d
Acanthella carteri



oroidin
Agelas oroides

Scheme 1. Structure formulas and indexing of pyrroles (top and left; C_3H_5 refers to 2-propenyl, that is, the allyl substituent), and sources of naturally occurring bromopyrroles[1,8,9] (center and bottom right).

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enzymes, however, shows similarly favorable characteristics as sustainable oxidation catalyst as the $V_{Br}PO(AnI)$.[11,12,18]

The existing knowledge about oxidative transformations catalyzed by bromoperoxidases[14,19,20] covers kinetic data on the oxidation of bromide,[13] oxidative transformations of chloride and thiocyanate,[11,21,22] singlet dioxygen formation,[23] sulfoxidation,[24] oxidation of alkenes,[25] and bromocyclization of terpenols on an analytical scale.[19] To us, this information did not suffice to extract parameters suitable to prepare bromopyrroles in a project on biomimetic synthesis of marine natural products (Scheme 1). We therefore chose to determine parameters that are relevant to synthesize bromopyrroles in ocean water-like media, using the bromoperoxidase I from *A. nodosum* as oxidation catalyst. To relate mechanistic issues with aspects of natural product chemistry, we selected 2-acceptor-substituted pyrroles as reporter substrates. The major results from the study show that selective mono- and dibromination of pyrroles, such as **1**, is feasible in yields of up to 91% and quantities up to 1.5 mmol, using the $V_{Br}PO(AnI)$ as oxidation catalyst. This method enabled us to prepare monobromopyrroles **2a**–**f**, and two marine natural products that is, methyl 4,5-dibromopyrrole-2-carboxylate (**3a**)[8] (from *Agelas oroides*) and 4,5-dibromopyrrole-2-carboxamide (**3d**)[8] (from *Acanthella carteri*) under biomimetic conditions.

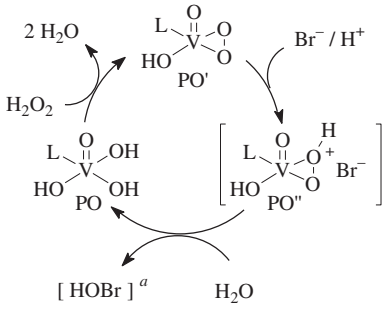
2. Results and interpretation

2.1. Enzyme purification and catalytic activity

Bromoperoxidase isoenzyme I [$V_{Br}PO(AnI)$] was isolated from lyophyllized *A. nodosum* according to an existing procedure.[26] The enzyme was stored in tris-(hydroxymethyl)-aminomethane [Tris, that is, 2-amino-2-(hydroxymethyl)-1,3-propanediol]-buffered stock solutions (pH 9.0) at 4 °C. The enzymatic activity was determined with the triiodide assay.[27] In this test, iodide is oxidized into triiodide, which is monitored in time-dependent photometric assay. The enzymatic activity is reported in units. One unit (1 U) thereby refers to the amount of enzyme necessary for turning over 1 μmol of substrate per minute. If the substrate is iodide, the associated enzymatic activity is abbreviated as U_T. The second parameter relevant for catalysis is the specific activity, which refers to the number of units per mg of the enzyme, that is, $U_T\ mg^{-1}$. Activity of bromoperoxidase preparations used in this study ranged between 526±11 and 611±6 $U_T^0\ mg^{-1}$ (in phosphate buffer, pH 6.2).

The triiodide assay provides information about purity of $V_{Br}PO(AnI)$-preparations, but no mechanistic details dealing with enzymatic reactivity under turnover conditions. To gain insight into the mechanism of bromoperoxidase-catalyzed oxidation, and substrate affinity in media similar to those applied in synthesis (vide infra), we performed a steady-state kinetic analysis in solutions of different ionic strengths (*I*).[28] The analytical method used for rate analysis under saturation conditions is the monochlorodimedone (2-chlorodimedone or MCD)-assay.[21,29,30] In the MCD-assay, the time-dependent decrease of the MCD-absorbance at λ_{max}=290 nm is correlated with the rate of 2-bromo-2-chlorodimedone formation. Monochlorodimedone bromination thereby occurs with similar rate as bromine is generated from bromoperoxidase-catalyzed bromide oxidation. Activity values determined with the MCD-assay, which are abbreviated as U_{MCD}^0, are smaller than the U_T^0-values, because iodide oxidation occurs faster than bromide oxidation.[26] Activities of $V_{Br}PO(AnI)$-preparations in the MCD-assay ranged between 127±4 and 171±2 $U_{MCD}^0\ mg^{-1}$ (pH 6.5, phosphate buffer).

To conduct steady-state-kinetic analysis, rates of enzymatic bromide oxidation under saturation conditions were determined for hydrogen peroxide concentrations that gradually were increased from 0.02 mM to 1.00 mM, as bromide concentrations were fixed between 1.00 mM and 400 mM. All kinetic experiments were performed in phosphate buffer at pH 5.9, to compare our results with reference data from the literature (vide infra). For numerical analysis, we, like others, applied the bi–bi–ping–pong model for extracting kinetic parameters from velocities and substrate concentrations. According to a bi–bi–ping–pong mechanism (Cleland-notation),[28] the $V_{Br}PO(AnI)$ (in short: PO) forms in a bimolecular reversible reaction an adduct PO′ by addition of one substrate from a pair of competing reactants. The reactant, that is, left from the first step binds to PO′ in the second, which again is assumed to be a reversible bimolecular reaction (Scheme 2). The complex PO″, which is formed from the enzyme and the two substrates, hydrolyzes in a non rate determining step to give the product of bromoperoxidase-catalyzed oxidation and the resting state of the enzyme, that is, PO. From the hydrogen peroxide- and the bromide-concentration dependence of the rate of enzymatic oxidation ($v_{oxidation}$, Eq. 5), we derived with suitable correlations the maximum velocity of the enzymatic reaction (V_{max}), Michaelis-parameters $K_m^{H_2O_2}$ and $K_m^{Br^-}$, and the constant $K_i^{Br^-}$, which describes competitive enzyme inhibition caused by bromide (Scheme 2, Table 1).[13,21] The quality of the fits provided no evidence for supplementing this model (Supplementary data).[28]



PO + H_2O_2 $\xrightleftharpoons{K_m^{H_2O_2}}$ PO' + 2 H_2O (eq. 1)

PO' + Br^- $\xrightleftharpoons{K_m^{Br^-}/H^+}$ PO" (eq. 2)

PO" $\longrightarrow$ PO + HOBr (eq. 3)

PO + Br^- $\xrightleftharpoons{K_i^{Br^-}}$ PO'" (eq. 4)

Scheme 2. Proposed mechanism for bromide oxidation, catalyzed by the vanadate(V)-dependent bromoperoxidase [$V_{Br}PO(AnI)$] (top; *An*=*Ascophyllum nodosum*; PO=peroxidase; PO′=peroxo form of the peroxidase; PO″=bromide adduct of the PO′; [a] assumed primary product of bromide oxidation; see text), and kinetic model for numerical analysis of the rate of bromide oxidation in the BPO-catalyzed reaction according to Eq. 5.[28]

Table 1
Steady state kinetic parameters for $V_{Br}PO(AnI)$-catalyzed of Br^- (pH 5.9, 22±0.5 °C)

Entry	I^a/M	NaCl/mM	V_{max}/U mg^{-1}	$K_m^{Br^-}$/mM	$K_m^{H_2O_2}$/μM	$K_i^{Br^-\,b}$/mM
1[c]	0.80	—	85±4	3.7±0.3	55±6	255
2[d]	1.65	—	107±7	2.9±0.3	75±7	557
3[d]	1.65	10	130±12	4.0±0.6	58±8	630

[a] Ionic strength was adjusted with Na_2SO_4.
[b] Due to comparatively large error bars in the correlation used to derive $K_i^{Br^-}$, standard deviations (±30–60%) for this constant were omitted from Table 1.
[c] 127±4 $U_{MCD}^0\ mg^{-1}$.
[d] 172±2 $U_{MCD}^0\ mg^{-1}$.

An analysis of enzyme kinetic data shows that Michaelis-constants for $K_m^{H_2O_2}$ at ionic strengths of 0.80 and 1.65 M are by two orders of magnitude smaller than $K_m^{Br^-}$. In terms of substrate affinity this result implies that hydrogen peroxide binds stronger to the enzyme than bromide. We therefore concluded that the peroxide is the first substrate to add to the active vanadate site. Condensation of hydrogen peroxide and vanadate(V) provides, from what is known from bromoperoxidase model chemistry,[16] a peroxo-loaded enzyme, that is, PO′ (Scheme 2). The bromide is second to interact with the active site, which, however, already exists in the peroxo form PO′. There is strong evidence from the literature, that the intermediate that finally oxidizes bromide, that is, PO″, is a hydroperoxy complex.[31,32] For reasons given below, we believe that PO″ affords hypobromous acid and the enzyme in resting state PO. Hypobromous acid gives in an aqueous solution of bromide an equilibrium mixture composed predominantly of tribromide, bromine, besides minor amounts of the starting acid.[33]

$$v_{oxidation} = \frac{\dfrac{V_{max}}{\left(1+\dfrac{K_m^{Br^-}}{[Br^-]}\right)}[H_2O_2]}{K_m^{H_2O_2}\dfrac{\left(1+\dfrac{[Br^-]}{K_i^{Br^-}}\right)}{\left(1+\dfrac{K_m^{Br^-}}{[Br^-]}\right)}} + [H_2O_2] \qquad \text{(eq. 5)}$$

The ratio of $K_m^{Br^-}/K_m^{H_2O_2}$ provides insight into relative substrate affinity of the applied bromoperoxidase under turnover conditions. These data compare reasonably well to reference $K_m^{Br^-}/K_m^{H_2O_2}$-ratios from the literature, referring to ionic strengths between 0.55 M and 0.80 M.[13,21] The absolute values of Michaelis-constants $K_m^{Br^-}$ and $K_m^{H_2O_2}$ determined in this study, however, were smaller than the references, thus indicating a higher substrate affinity of bromoperoxidase preparations used in this work compared to those applied previously.

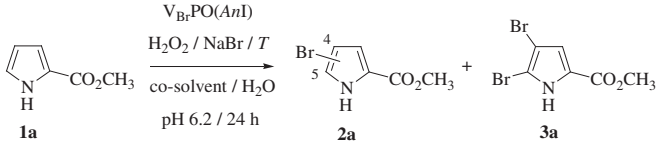
The effect of salinity on bromoperoxidase reactivity is small. As the ionic strength increases from 0.80 M (ocean water)[34] to 1.65 M, the affinity of the enzyme for hydrogen peroxide binding decreases by a factor of 1.4 (Table 1, entry 2). Addition of chloride (10 mM) reverts this effect to some extend (Table 1, entries 1 and 3). Raising the ionic strength to 1.65 M has no significant effect on bromide binding.

2.2. Reaction parameters for oxidation catalysis

We selected methyl 1*H*-pyrrole-2-carboxylate (**1a**) as substrate to find parameters suitable for pyrrole bromination in bromoperoxidase-catalyzed oxidations. Heteroarene **1a** undergoes substitution of bromine for hydrogen, if treated with hypobromous acid, bromine, or tribromide in the absence of a Lewis acid. The substrate also provides a diagnostic mixture of mono- and disubstitution products **2a** and **3a**, which are sufficiently stable for quantitative analysis via gas chromatography, and purification by column chromatography for determining the site of aromatic substitution by NMR-spectroscopy.[2,3] Since pyrrole-2-carboxylic acid in nature is formed from the amino acid metabolism,[1] the results are relevant for providing insight into biomimetic synthesis of naturally occurring bromopyrroles.[8]

2.2.1. Effect of substrate addition, co-solvent, and temperature. For synthetic application (Table 2), we replaced the phosphate buffer used in the kinetic experiments by morpholine-4-ethanesulfonic acid (MES, pH 6.2). This modification extended the half-life time of $V_{Br}PO(AnI)$-activity by a factor of 44 to 8.8 days.[35]

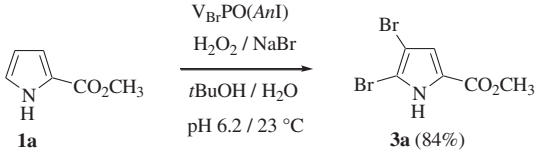
Table 2
Summary of relevant reaction parameters for effective methyl pyrrole-2-carboxylate bromination



Entry	Co-solvent	T/°C	Conv. **1a**[a]/%	pH^{final}[b]	Activity/ U_T^{final} mg^{-1}[c]	**2a**/% (4/5)	**3a**/%
1	^{t}BuOH	23	quant.[d]	6.6	117	99 (94/6)	—[e]
2	**^{t}BuOH**	**23**	**quant.**[f]	**6.6**	**43**	**75 (87/13)**	**16**[g]
3	**^{t}BuOH**	**23**	**84**	**6.6**	**267**	**64 (77/23)**	**7**
4	^{t}BuOH	40	80	6.6	196	67 (78/22)	6
5	^{t}BuOH[h]	40	81	6.5	67	64 (77/23)	5
6	^{t}BuOH	60	72	6.4	—[i]	53 (75/25)	4
7	1,4-Dioxane	23	80	6.6	265	63 (75/25)	8
8	1,4-Dioxane	40	80	6.5	209	64 (77/23)	7
9	CH_3CN	23	80	6.5	318	60 (80/20)	7
10	CH_3CN	40	76	6.5	247	60 (78/22)	7

Entries in bold refer to recommended conditions for application in synthesis.
[a] Unless otherwise stated, continuous addition (syringe pump, 8 h) of a solution of H_2O_2 (0.825 M, 1.1 equiv) and NaBr (0.750 M, 1.0 equiv) in aqueous MES-buffer (pH 6.2)/co-solvent=75/25% (v/v); 1.5 mmol of **1a** were used as starting material except of entry 2 (0.75 mmol).
[b] pH electrode.
[c] Initial enzyme activity 526 U_T^0 mg^{-1} (entry 1) and 558 U_T^0 mg^{-1} (entries 2–10).
[d] $c_{\mathbf{1a}}^0$ = 7.2 mM (36 μmol of **1a**), H_2O_2 addition in 3 batches every 30 min; total amount of substrates: 1.0 equiv of H_2O_2 and 3.0 equiv of NaBr.
[e] Not detected.
[f] H_2O_2 (2.2 equiv) and 3.0 equiv of NaBr in MES-buffer continuously added over a period of 8 h.
[g] Additional product: 3% of methyl 3,4-dibromopyrrole-2-carboxylate.
[h] NaCl (0.5 M) added.
[i] No final bromoperoxidase activity detected.

(*i*) Substrate/enzyme-ratio. To achieve quantitative substrate conversion in synthetic scale experiments (1.5 mmol of **1a**), the amount of the bromoperoxidase was doubled to 35 U_T (17 U_T for 36 μM of **1a**). Hydrogen peroxide (2.2-fold) and sodium bromide (3.0-fold) were for similar reasons used in excess (Table 2, entries 1 and 2). On the assumption that both active sites of the 120 kDa $V_{Br}PO(AnI)$-homodimer were occupied by vanadate, 35 U_T corresponds to a substrate/enzyme-ratio of 36 μmol % (Table 2, entries 2–10). At elevated concentration of hydrogen peroxide and bromide, regioselectivity of monobromide formation declines and dibromide **3a** is formed as additional product. The dibromide, which is a naturally occurring constituent from the marine sponge *A. oroides*,[8] becomes the major product, as the amounts of $V_{Br}PO(AnI)$ (34.6 U_T), sodium bromide, and hydrogen peroxide were doubled (Scheme 3). Methyl 4,5-dibromopyrrole-2-carboxylate **3a** shows an interesting antiplasmoidal, cytotoxic, and electrophysiologic profile and therefore has attracted attention of medicinal chemists.[36,37]



Scheme 3. Synthesis of naturally occurring (*A. oroides*) brominated pyrrole **3a** [conditions: 63 μmol % of $V_{Br}PO(AnI)$ (34.6 U_T), 2.2 equiv of NaBr, and 2.2 equiv of H_2O_2].

Selective monobromination of **1a** in bromoperoxidase-catalyzed oxidations was feasible, but required to cut the amount of hydrogen peroxide and sodium bromide by half. Under such conditions, the maximum yield of **2a** remained at 36%. Routine analysis for peroxides with the photometric Huber–Fröhlke test[38] was negative by the time pyrrole bromination came to an end.

(*ii*) Hydrogen peroxide concentration and the mode of hydrogen peroxide administration. Continuous addition within eight hours of a solution containing aqueous hydrogen peroxide (0.825 M) and morpholine-4-ethanesulfonic acid (MES) to a solution of the bromoperoxidase and pyrrole **1a** gave 64% of monobromopyrrole **2a** and 7% of dibromide **3a**. If referenced versus substrate utilization, the yields of target products **2a**–**3a** corresponded to a selectivity of 71% for hydrogen peroxide consumption and 78% for the use of bromide (Table 2, entry 3). Discontinuous hydrogen peroxide addition (c^0=0.825 M), either in a single batch or in up to three regular intervals, reduced conversion of **1a** and thus lowered the yields of bromopyrroles **2a** and **3a** (Supplementary data). The use of higher hydrogen peroxide strengths than 0.825 M caused bromoperoxidase activity to rapidly decline, without providing substantial amounts of brominated pyrroles **2a**–**3a**.

(*iii*) Organic solvent, temperature, and buffer capacity. *tert*-Butanol, 1,4-dioxane, and acetonitrile formed homogeneous mixtures with the MES-buffer and were similarly suited as co-solvent for pyrrole bromination (Table 1, entries 3, 7, 9). The use of dimethyl sulfoxide, acetone, and dimethyl carbonate as co-solvent for storing the enzyme at 4 °C caused bromoperoxidase activity to rapidly cease. To our surprise, bromination of methyl pyrrole-2-carboxylate **1a** occurred even in the absence of an organic co-solvent (62% **2a**, 5% **3a**). The efficiency of pyrrole bromination increased as the temperature rose from 23 °C to 40 °C. At 60 °C, however, the yields of bromopyrroles **2a**–**3a** remained below values obtained at lower temperatures, and the bromoperoxidase activity was entirely lost in the course of the experiment without providing quantitative turnover of substrate **1a** (Table 2, entries 3–10). For reasons of sustainability, we chose room temperature (~23 °C) as standard, to conduct bromination of 2-acceptor-substituted pyrroles **1b**–**f**.

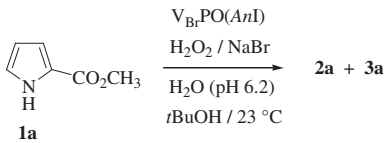
To prepare 1.5 mmol of bromopyrrole **2a** or 0.75 mmol of dibromide **3a**, we found that 10.0 mmol of morpholine-4-ethanesulfonic acid were adequate to maintain the pH of reaction mixtures close 6.2 to conserve maximum bromoperoxidase activity under turnover conditions.

(*iv*) Bromoperoxidase activity. Under appropriate conditions, up 50% of bromoperoxidase activity was retained in oxidations dealing with methyl pyrrole-2-carboxylate bromination (cf. Table 2, entry 3). This activity was used to brominate additional substrate **1a**. With every run, the efficiency for turning over **1a** decreased, leading to 34% of **2a** in a second, 10% in a third, and 8% in a fourth cycle (Supplementary data). If 17 U_T instead of the recommended amount of 35 U_T were used, the bromoperoxidase activity, however, was already lost in the first run.

(*v*) Controls. In the absence of the $V_{Br}PO(AnI)$, no bromination of substrate **1a** occurs. Addition of ammonium metavanadate does not change this situation. Treatment of pyrrole **1a** with aliquots of bromide-free hypobromous acid (c^0=0.11 M), bromine, or tetrabutylammonium tribromide (c^0=0.06 M for both) in aqueous *tert*-butanol (23 °C) provides mixtures of mono- and dibromides **2a**–**3a**. The yields of bromopyrrole **2a** formed from the reaction between pyrrole **1a** and tetrabutylammonium tribromide (66%, 88/12-mixture of 4/5-isomers) were higher than those starting from bromine or bromide-free hypobromous acid (7–8%, 4-isomer in both instances). The yields of dibromide **3a** formed from **1a** increased along the series of applied bromination reagents from tetrabutylammonium tribromide (1%) via hypobromous acid (5%) to bromine (12%). The reaction between pyrrole **1a** and bromine furthermore provided methyl-3,4,5-tribromopyrrole-2-carboxylate **7a** in 8% yield.

2.2.2. Effect of NaBr preincubation. Methyl pyrrole-2-carboxylate bromination becomes more efficient and selective, as $V_{Br}PO(AnI)$-solutions were preincubated with up to 0.5 M of bromide (compare

Table 3
Effect of bromide on selectivity of methyl pyrrole-2-carboxylate bromination



1a

Entry	$c^0_{Br^-}$/M	Conv. **1a**[a]/%	Activity/U_T^{final} mg^{-1}[b]	**2a**/% (4/5)	**3a**/%
1	—	83	313	68 (76/24)	7
2	0.1	93	339	71 (87/13)	10
3	0.25	91	299	76 (88/12)	6
4	0.5	92	353	79 (90/10)	6
5	1.0	84	197	70 (88/12)	2

[a] pH^{final}=6.5 for entries 1–5.
[b] 558 U_T^0 mg^{-1}, ±5% experimental error.

entries 1 and 4 in Table 3). An inhibitory effect of the halide, as expected on the basis of steady state kinetic data (cf. $K_i^{Br^-}$ in Table 1), was not observed under conditions used for turning over **1a**.

2.2.3. Terminal oxidants other than H_2O_2. Addition of neat urea-hydrogen peroxide (UHP) in a single batch to a standard solution containing sodium bromide, pyrrole **1a**, and the bromoperoxidase (for standard conditions, see Table 2, entry 3) afforded monobromide **2a** (51%) and 4,5-dibromide **3a** (3% for 67% conversion of **1a**). The pH of the solution in this experiment rose from 6.2 to 6.4 during substrate turnover. About 4% of the initial bromoperoxidase activity (588 U_T^0 mg^{-1}) were left in this experiment, as pyrrole bromination came to a standstill.

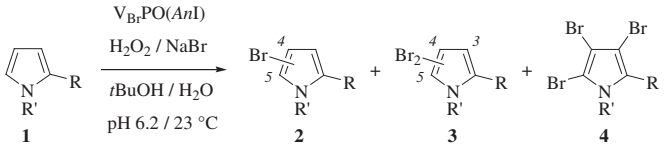
Addition of oxone® (2 $KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$) to a standard reaction mixture caused a drop in pH from 6.2 to ~4. This effect could be moderated but not entirely inhibited, by raising the buffer concentration. Quantitative conversion of pyrrole **1a** under such conditions was not attainable. The yield of bromopyrrole **2a** (25%) in oxidations mediated by oxone® was similar to a value obtained in the absence of the $V_{Br}PO(AnI)$, under otherwise identical conditions. The use of sodium carbonate perhydrate caused a pH shift from 6.2 to >10. Conversion of substrate **1a** in this case did not exceed ~6%. Addition of aqueous hydrochloric acid, to adjust pH of the mixture to 6.2, reconstituted 19% of the original bromoperoxidase activity (triiodide test).

2.3. Synthesis of bromopyrroles

Allyl or pentyl esters of pyrrole-2-carboxylic acid (compounds **1b** and **c**) provided mixtures of mono- and dibromides **2b**/**2c**–**3b**/**3c** (Table 4, entries 1 and 2), in solutions containing the bromoperoxidase, and a threefold excess of hydrogen peroxide and sodium bromide. Similarly, pyrrole-2-carboxamide **1d**[39–41] and carbonitrile **1e**[42,43] furnished products of oxidative bromination if treated under standard conditions with bromoperoxidase (Table 4, entries 3 and 4).

Pyrrole itself afforded under standard conditions a dark viscous resin that could not be further purified into low molecular weight products. *N*-Phenylpyrrole[44] and methyl thiophene-2-carboxylate were almost quantitatively recovered, if treated with the bromoperoxidase, sodium bromide, and hydrogen peroxide. The yield of

Table 4
Bromination of substituted pyrroles



Entry	1–4	R	R′	Conv. 1/%[a]	2/% (4/5)	3/%[b]	4/%
1	**a**	CO_2CH_3	H	95	53 (81/19)	33	—[c]
2	**b**	$CO_2C_3H_5$	H	quant.	24 (>98/2)	40	27
3	**c**	$CO_2C_5H_{11}$	H	53	26 (81/19)	17	—[c]
4	**d**	$CONH_2$	H	71	42 (71/89)	9	—[c]
5	**e**	CN	H	quant.	40 (>98/2)	—[c]	35
6	**f**	CO_2CH_3	C_2H_5	19[d]	9 (>98/2)	—[c]	—[c]

[a] 34.6 U_T corresponds to 32 μmol % of $V_{Br}PO(AnI)$ [611 U_T^0 mg^{-1}, except of entry 5 (558 U_T^0 mg^{-1})], 1.5 mmol of **1**, 3.3 equiv of H_2O_2 3.0 equiv of NaBr.
[b] 4,5-substitution.
[c] Not detected (^{1}H NMR).
[d] 21.5 U_T corresponds to ~32 μmol % of $V_{Br}PO(AnI)$ (611 U_T^0 mg^{-1}), 0.9 mmol of **1f**, 3.3 equiv of H_2O_2 and 3.0 equiv of NaBr.

methyl 4-bromo-1-ethylpyrrole-2-carboxylate (**2f**)[45] in $V_{Br}PO(AnI)$-catalyzed oxidations under standard conditions remained low. Routine triiodide tests for bromoperoxidase activity at the end of the experiments were positive (Supplementary data).

3. Concluding remarks

2-Acceptor-substituted 1*H*-pyrroles undergo electrophilic (hetero)aromatic bromination in aqueous solutions of bromide and hydrogen peroxide.[46] Bromide oxidation thereby occurs at ambient temperature and pH 6.2. The oxidation is catalyzed by the vanadate(V)-dependent bromoperoxidase I (isoenzyme I) from *A. nodosum* (PDB-code 1QI9),[47] to furnish hypobromous acid, which then equilibrates in the bromide containing brine, to give bromine and tribromide.[33,48] 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),[49,50] and therefore cannot account for the electrophilic behavior of the bromination reagent that transforms pyrroles into bromopyrroles. Among the reactants formed in $V_{Br}PO(AnI)$-catalyzed oxidation, only molecular bromine shows adequate chemical reactivity to explain the observed selectivity in pyrrole bromination.

Although the substrates chosen to determine parameters relevant for synthesis of bromopyrroles with $V_{Br}PO(AnI)$ reflect interests from our laboratory, we believe that bromoperoxidase-catalyzed oxidations have the potential to become a quite general supplement to traditional brominations of π-nucleophilic substrates, wherever handling of molecular bromine shall be avoided for toxicological reasons. To exemplify the relevance of the present study for understanding biomimetic synthesis of naturally occuring organobromines it is instructive to recall that methyl 4,5-dibromopyrrole-2-carboxylate (**3a**) (from *A. oroides*) and carboxamide **3d** (from *A. carteri*[39]) were isolated in earlier studies from marine sponges. To us, these results point to a systematic for predicting selectivity in brominations induced by bromoperoxidase-catalyzed oxidations, which is being investigated at the moment in more detail in this laboratory.

The bromoperoxidase used in this study is available from an alga that grows abundantly along shorelines of North Atlantic coasts. In view of the quality of this catalyst it is instructive to remember that bromoperoxidases also exist in other organisms, such as *Corallina officinalis*, *C. vancouveriensis*, *Laurencia pacifica*, *Macrocystis pyrifera*, *Plocamium cartilagineum*, and *Fucus distichus*.[11,12,14] These enzymes offer potential for improving reactivity and selectivity in oxidation catalysis, and to take on the challenge to find substrate specificity for carbon–bromine bond formation in synthesis of marine natural products.[51]

4. Experimental

4.1. General remarks

For instrumentation, comments on laboratory standards, and details of compound preparations, refer to the Supplementary data. $V_{Br}PO(AnI)$ was isolated from *A. nodosum* collected in April 2004 and 2009 (France, 48° 43′ N, 3° 58′ W) according to a published procedure.[26]

4.2. General method for pyrrole bromination in bromoperoxidase-catalyzed reactions

A solution of H_2O_2 (3×2.0 mL, 0.825 M) and NaBr (3×154.4 mg, 1.50 mmol) in MES-buffer (500 mM, pH 6.2) was added every day with a syringe pump (8 h, 0.004 mL/min) to a solution of substrate **1** (1.50 mmol) and $V_{Br}PO(AnI)$ (34.6 U_T, ~0.032 mmol%) in MES-buffer (500 mM, pH 6.2, 20.0 ml) and ^{t}BuOH (6.6 mL) [for: **1f** 3× H_2O_2 (2.0 mL, 0.511 M) and NaBr (95.7 mg, 0.93 mmol) in MES-buffer 500 mM to a solution of **1f** (0.93 mmol) and $V_{Br}PO(AnI)$ (21.5 U_T, ~0.032 mmol%) in MES-buffer (500 mM, pH 6.2, 10.1 ml) and ^{t}BuOH (4.1 mL)]. The reaction mixture was stirred at 23 °C for 3 days. The aqueous layer was extracted with Et_2O (3×20 mL). Combined organic extracts were dried ($MgSO_4$). The solvent was removed under reduced pressure (14 mbar, 40 °C) to afford a product mixture, which was analyzed by ^{1}H NMR and GC, using pentachlorobenzene (^{1}H NMR) as an internal standard in comparison to spectral data from authentic references. New compounds were purified via column chromatography and fully characterized. pH and bromoperoxidase activity (triiodide assay) were determined from the aqueous layer.

*4.2.1. Bromination of methyl 1H-pyrrole-2-carboxylate (**1a**).* According to general method in Section 4.2. Yield: 328 mg, rose solid; 50/12/38-mixture of $\mathbf{2a}_{4-Br}$/$\mathbf{2a}_{5-Br}$/$\mathbf{3a}_{4,5-Br_2}$-isomers. Methyl 4-bromo-1*H*-pyrrole-2-carboxylate[2] ($\mathbf{2a}_{4-Br}$). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 3.87 (s, 3H), 6.88–6.89 (m, 1H), 6.94–6.95 (m, 1H), 9.39 (br s, 1H, NH). MS (EI) *m*/*z* 205 (77), 203 (77), 173 (100), 171 (100), 146 (26), 144 (26), 119 (18), 117 (18), 64 (47). Methyl 5-bromo-1*H*-pyrrole-2-carboxylate[3] ($\mathbf{2a}_{5-Br}$). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 3.86 (s, 3H), 6.21–6.22 (m, 1H), 6.82–8.83 (m, 1H), 9.48 (br s, 1H, NH). MS (EI) *m*/*z* 205 (68), 203 (68), 173 (100), 171 (100), 146 (23), 144 (23), 119 (30), 117 (30), 64 (48). Methyl 4,5-dibromo-1*H*-pyrrole-2-carboxylate[2] ($\mathbf{3a}_{4,5-Br_2}$). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 3.90 (s, 3H), 6.87 (d, 1H, *J*=2.9 Hz), 10.06 (br s, 1H, NH). MS (EI) *m*/*z* 285 (27), 283 (58), 281 (32), 253 (48), 251 (100), 249 (52), 226 (6), 224 (14), 222 (6), 199 (6), 197 (15), 195 (8), 144 (14), 142 (14). 611 U_T^0 mg^{-1}, 31 U_T^{final} mg^{-1}, pH^{final} 6.5.

*4.2.2. Bromination of prop-2-enyl 1H-pyrrole-2-carboxylate (**1b**).* According to general method in Section 4.2. For isomer separation see also Supplementary data. Yield: 445 mg, colorless solid; 26/4/30-mixture of $\mathbf{2b}_{4-Br}$/$\mathbf{3b}_{4,5-Br_2}$/$\mathbf{4b}_{3,4,5-Br_3}$-isomers. Prop-2-enyl 4-bromo-1*H*-pyrrole-2-carboxylate ($\mathbf{2b}_{4-Br}$). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 4.78 (d, 2H, *J*=5.6 Hz), 5.28 (dd, 1H, *J*=10.6, 0.9 Hz), 5.38 (dd, 1H, *J*=17.1, 1.3 Hz), 5.95–6.04 (m, 1H), 6.92–6.93 (m, 1H), 6.95–6.96 (m, 1H), 9.52 (br s, 1H, NH). ^{13}C NMR ($CDCl_3$, 150 MHz) δ 65.5, 98.0, 117.3, 118.7, 123.0, 123.1, 132.0, 160.3. MS (EI) *m*/*z* 231 (46), 229 (46), 174 (100), 172 (100), 146 (23), 144 (23), 119 (18), 117 (18), 93 (11), 65 (37). Prop-2-enyl 4,5-dibromo-1*H*-pyrrole-2-carboxylate ($\mathbf{3b}_{4,5-Br_2}$). Colorless crystalline solid, mp 103 °C, R_f=0.36

for pentane/acetone=5:1 (v/v). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 4.78 (dd, 2H, J=4.5, 1.8 Hz), 5.30 (dd, 1H, J=10.5, 1.0 Hz), 5.39 (dd, 1H, J=17.2, 1.5 Hz), 5.95–6.02 (m, 1H), 6.92 (d, 1H, J=2.8 Hz), 9.82 (br s, 1H, NH). ^{13}C NMR ($CDCl_3$, 150 MHz) δ 65.8, 100.9, 107.4, 118.3, 119.0, 123.8, 131.8, 159.6. MS (EI) m/z 311 (35), 309 (74), 307 (38), 294 (6), 292 (11), 290 (6), 254 (52), 252 (100), 250 (52), 230 (20), 228 (20), 226 (14), 224 (26), 222 (14), 199 (21), 197 (44), 195 (23), 149 (71), 144 (24), 142 (24). Anal. Calcd for $C_8H_7Br_2NO_2$ (308.96): C, 31.10; H, 2.28; N, 4.53; Found: C, 31.37; H, 2.33; N, 4.56. Prop-2-enyl 3,4,5-tribromo-1*H*-pyrrole-2-carboxylate (**4b**$_{3,4,5-Br_3}$). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 4.85 (d, 2H, J=5.6 Hz), 5.31 (dd, 1H, J=10.6, 0.8 Hz), 5.47 (dd, 1H, J=17.2, 1.3 Hz), 5.95–6.04 (m, 1H), 10.22 (br s, 1H, NH). ^{13}C NMR ($CDCl_3$, 150 MHz) δ 66.2, 105.7, 106.7, 107.2, 119.0, 122.0, 131.5, 159.0. MS (EI) m/z 341 (22), 399 (64), 387 (65), 385 (23), 334 (33), 332 (96), 330 (100), 328 (37), 310 (22), 308 (49), 306 (32), 292 (18), 290 (34), 288 (16), 279 (14), 277 (40), 275 (40), 273 (13), 198 (26), 196 (51), 194 (25). 611 U_T^0 mg^{-1}, 6 U_T^{final} mg^{-1}, pH^{final} 6.6.

*4.2.3. Bromination of pentyl 1H-pyrrole-2-carboxylate (**1c**).* According to general method in Section 4.2. For isomer separation see also the Supplementary data. Yield: 353 mg, dark yellow oil; 49/12/39-mixture of **2c**$_{4-Br}$/**2c**$_{5-Br}$/**3c**$_{4,5-Br_2}$-isomers. Pentyl 4-bromo-1*H*-pyrrole-2-carboxylate (**2c**$_{4-Br}$). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 0.92 (t, 3H, J=7.1 Hz), 1.35–1.41 (m, 4H), 1.72–1.74 (m, 2H), 4.26 (t, 2H, J=6.8 Hz), 6.88–6.89 (m, 1H), 6.93–6.94 (m, 1H), 9.23 (br s, 1H, NH). ^{13}C NMR ($CDCl_3$, 150 MHz) δ 14.1, 22.5, 28.3, 28.7, 65.1, 97.9, 116.8, 122.6, 123.5, 160.6. MS (EI) m/z 261(22), 259 (20), 189 (100), 187 (100), 173 (94), 171 (92), 146 (19), 144 (19), 93 (8), 65 (25). Pentyl 5-bromo-1*H*-pyrrole-2-carboxylate (**2c**$_{5-Br}$). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 0.92 (t, 3H, J=7.0 Hz), 1.36–1.39 (m, 4H), 1.71–1.73 (m, 2H), 4.26 (t, 2H, J=6.7 Hz), 6.21–6.22 (m, 1H), 6.82–6.83 (m, 1H), 9.25 (br s, 1H, NH). MS (EI) m/z 261 (15), 259 (15), 189 (100), 187 (100), 173 (85), 171 (89), 146 (17), 144 (12), 93 (8), 65 (22). Pentyl 4,5-dibromo-1*H*-pyrrole-2-carboxylate (**3c**$_{4,5-Br_2}$). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 0.92 (t, 3H, J=7.0 Hz), 1.36–1.39 (m, 4H), 1.71–1.73 (m, 2H), 4.26 (t, 2H, J=6.7 Hz), 6.88 (d, 1H, J=2.8 Hz), 9.50 (br s, 1H, NH). MS (EI) m/z 341 (10), 339 (21), 337 (11), 271 (51), 269 (100), 267 (53), 253 (55), 251 (99), 249 (54), 226 (7), 224 (15), 222 (7). ^{13}C NMR ($CDCl_3$, 150 MHz; mixture of **2c**$_{5-Br}$/**3c**$_{4,5-Br_2}$) δ 14.1, 22.5, 28.2, 28.5, 28.6, 65.0, 65.4, 100.8, 104.8, 106.8, 112.8, 116.7, 117.8, 124.3, 124.4, 159.9, 160.4. 611 U_T^0 mg^{-1}, 32 U_T^{final} mg^{-1}, pH^{final} 6.4.

*4.2.4. Bromination of 1H-pyrrole-2-carboxamide (**1d**).* According to general method in Section 4.2. Yield: 262 mg, colorless semi-solid; 59/24/17-mixture of **2d**$_{4-Br}$/**2d**$_{5-Br}$/**3d**$_{4,5-Br2}$-isomers. 4-Bromo-1*H*-pyrrole-2-carboxamide (**2d**$_{4-Br}$).[39] ^{1}H NMR (DMSO-d_6, 600 MHz) δ 6.83–6.84 (m, 1H), 6.96–6.97 (m, 1H), 7.09 (br s, 1H, NH_2), 7.57 (br s, 1H, NH_2) 11.76 (br s, 1H, NH). MS (EI) m/z 190 (97), 188 (97), 173 (100), 171 (100), 146 (21), 144 (21), 119 (20), 117 (20), 109 (15), 64 (58). 5-Bromo-1*H*-pyrrole-2-carboxamide (**2d**$_{5-Br}$).[40] ^{1}H NMR (DMSO-d_6, 600 MHz) δ 6.11–6.12 (m, 1H), 6.74–6.75 (m, 1H), 7.00 (br s, 1H, NH_2), 7.45 (br s, 1H, NH_2) 12.15 (br s, 1H, NH). MS (EI) m/z 190 (100), 188 (100), 173 (95), 171 (95), 146 (23), 144 (23), 119 (33), 117 (33), 64 (71). 4,5-Dibromo-1*H*-pyrrole-2-carboxamide (**3d**$_{4,5-Br_2}$).[41] ^{1}H NMR (DMSO-d_6, 600 MHz) δ 6.92 (s, 1H), 7.20 (br s, 1H, NH_2), 7.58 (br s, 1H, NH_2), 12.64 (br s, 1H, NH). MS (EI) m/z 270 (32), 268 (67), 266 (33), 253 (52), 251 (100), 249 (45), 226 (5), 224 (8), 222 (5), 199 (6), 197 (12), 195 (5), 173 (5), 171 (5). 611 U_T^0 mg^{-1}, 41 U_T^{final} mg^{-1}, pH^{final} 6.6.

*4.2.5. Bromination of 1H-pyrrole-2-carbonitrile (**1e**).* According to general method in Section 4.2. For isomer separation see also the Supplementary data. Yield: 333 mg, rose solid; 53/47-mixture of **2e**$_{4-Br}$/**4e**$_{3,4,5-Br_3}$-isomers. 4-Bromo-1*H*-pyrrole-2-carbonitrile (**2e**$_{4-Br}$). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 7.05–7.06 (m, 1H), 7.33–7.34 (m, 1H), 12.69 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6, 150 MHz) δ 95.5, 101.0, 113.6, 120.5, 124.9. MS (EI) m/z 172 (97), 170 (100), 145 (9), 143 (10), 91 (21), 64 (57). 3,4,5-Tribromo-1*H*-pyrrole-2-carbonitrile (**4e**$_{3,4,5-Br_3}$). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 14.10 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6, 150 MHz) δ 102.0, 103.5, 108.2, 109.0, 111.9. MS (EI) m/z 332 (30), 330 (93), 328 (100), 326 (33), 251 (13), 249 (27), 247 (12), 224 (9), 222 (19), 220 (9), 172 (10), 170 (10). Anal. Calcd for $C_5H_3BrN_2$ (169.99)/$C_5HBr_3N_2$ (328.78) (53/47): C, 24.50; H, 0.85; N, 11.42; Found: C, 24.22; H, 0.89; N, 11.23. 558 U_T^0 mg^{-1}, not detected U_T^{final} mg^{-1}, pH^{final} 7.0.

*4.2.6. Bromination of methyl 1-ethylpyrrole-2-carboxylate (**1f**).* According to general method in Section 4.2. Yield: 134 mg, yellowish liquid; 90/10-mixture of **1f**/**2f**$_{4-Br}$-isomers. Methyl 4-bromo-1-ethylpyrrole-2-carboxylate (**2f**$_{4-Br}$). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 1.39 (t, 3H, J=7.2 Hz), 3.81 (s, 3H), 4.35 (q, 2H, J=7.1 Hz), 6.83 (d, 1H, J=1.8 Hz), 6.90 (d, 1H, J=1.8 Hz). MS (EI) m/z 233 (97), 231 (100), 218 (27), 216 (29), 202 (55), 200 (61), 173 (41), 171 (38), 146 (12), 144 (12), 120 (26), 93 (24). 611 U_T^0 mg^{-1}, 53 U_T^{final} mg^{-1}, pH^{final} 6.0.

4.3. Synthesis of methyl 4,5-dibromopyrrole-2-carboxylate (**3a**$_{4,5-Br_2}$)

A solution of H_2O_2 (2.0 mL, 0.825 M) and NaBr (169.8 mg, 1.65 mmol) in MES-buffer (500 mM, pH 6.2) was added with a syringe pump (20 h, 1.67 μL/min) to a solution of substrate **1a** (0.75 mmol) and V_{Br}PO(*An*I) (125.6 μL, 34.6 U_T, 0.063 mmol%) in MES-buffer (500 mM, pH 6.2, 10.0 ml) and ^{t}BuOH (3.3 mL). The reaction mixture was stirred at 23 °C for 1 day. The aqueous layer was extracted with Et_2O (3×20 mL). Combined organic extracts were dried ($MgSO_4$). The solvent was removed under reduced pressure (14 mbar, 40 °C) to afford a product mixture, which was analyzed by ^{1}H NMR and GC, using pentachlorobenzene (^{1}H NMR) as an internal standard in comparison to spectral data from authentic references. For spectral data see Section 4.2.1. 611 U_T^0 mg^{-1}, 279 U_T^{final} mg^{-1}, pH^{final} 6.9.

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Supplementary data

Instrumentation, reagent specification, details about steady state kinetic data and analysis, experimental procedures, spectral and analytical data of new compounds. Supplementary data related to this article can be found online at doi:10.1016/j.tet.2011.04.010.

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