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Inhibition of Bovine Plasma Amine Oxidase by 1,4-Diamino-2-butenes and -2-butynes

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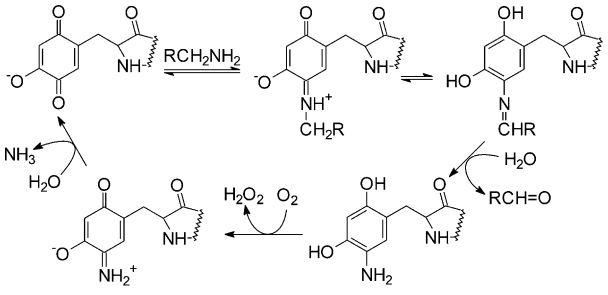
Abstract—Bovine plasma amine oxidase (BPAO) was previously shown to be irreversibly inhibited by propargylamine and 2-chloroallylamine. 1,4-Diamine versions of these two compounds are here shown to be highly potent inactivators, with IC_{50} values near 20 μM. Mono-*N*-alkylation or *N*,*N*-dialkylation greatly lowered the inactivation potency in every case, whereas the mono-*N*-acyl derivatives were also weaker inhibitors and enzyme activity was recoverable. The finding that the bis-primary amines 1,4-diamino-2-butyne (a known potent inhibitor of diamine oxidases) and *Z*-2-chloro-1,4-diamino-2-butene are potent inactivators of BPAO is suggestive of unexpected similarities between plasma amine oxidase and the diamine oxidases and implies that it may be unwise to attempt to develop selective inhibitors of diamine oxidase using a diamine construct.

Introduction

The copper-containing amine oxidases convert unbranched primary amines to the corresponding aldehydes by use of an active-site quinone-mediated transamination reaction. Based on the fairly recent elucidation of the structures of the quinone cofactors, there has been renewed interest in the mechanistic aspects of oxidative deamination and in the development of useful inhibitors. Most of the enzymes in this class utilize a 2,4,6-trihydroxyphenylalanine quinone (TPQ) cofactor derived from an active site tyrosine.[1,2] A different cofactor, lysine tyrosylquinone (LTQ), that represents an active site lysine–tyrosine cross-link,[3] is used by a sub-class of these enzymes, lysyl oxidases, responsible for oxidative deamination of the lysine side chains of the connective tissue proteins collagen and elastin.

The mechanism of the deamination half-reaction[4] involves formation of a Schiff base between quinone cofactor and substrate amine, isomerization of the 'substrate Schiff base' to a 'product Schiff base' with reduction of the quinone (the key transamination step), and hydrolysis of the product Schiff base (see Scheme 1 for abbreviated mechanism using TPQ). Subsequently, the reduced cofactor is converted to the starting quinone at the expense of reduction of O_2 to H_2O_2, with release of ammonia. Across the plant and animal kingdoms, there are several sub-classes of copper amine oxidases with different tissue distribution and substrate preferences. The mammalian plasma amine oxidases, the human form of which is often known as the soluble semicarbazide-sensitive amine oxidase (SSAO),[5] prefer to metabolize arylalkylamines including benzylamine, the phenethylamine neurotransmitters, and exogenous amine drugs. On the other hand, a family of plant enzymes and mammalian enzyme found in kidney, intestines, and placenta, referred to as diamine oxidases (DAOs), prefers to metabolize aliphatic 1,4- and 1,5-diamines and the neurotransmitter histamine. It is believed that these latter enzymes contain a cation binding site located at an appropriate distance (~10 Å) from the reactive cofactor carbonyl,[6] such that electro-



Scheme 1.

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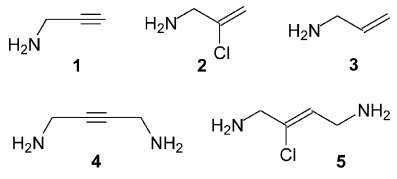
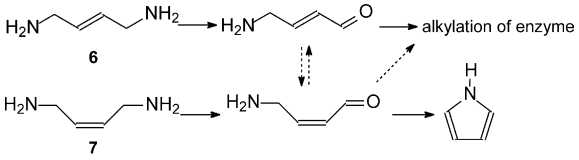
static binding (and possibly hydrogen-bonding) of one protonated terminus of the diamine (or imidazolium ring in histamine) positions the other primary amine terminus properly for Schiff base formation with the cofactor.

Based on work mainly directed at inactivating pyridoxal phosphate (PLP)-dependent transamination enzymes, Abeles and co-workers reported nearly 30 years ago that amines bearing an unsaturated C–C bond at the β-position could inactivate bovine plasma amine oxidase (BPAO).[7,8] Propargylamine (**1**) and 2-chloroallylamine (**2**) were found to be inactivators, though allylamine (**3**) was found to be only a substrate with no inactivation tendency. Also, the propargylic diamine analogue 1,4-diamino-2-butyne (**4**) was reported to exhibit 50% inactivation of BPAO at 2.5 mM in 0.23 min[8] despite little knowledge that diamines are substrates for this enzyme. However, few details were revealed by these early studies. More recently, Frebort and co-workers reported that **4** is a potent mechanism-based inactivator of pea DAO[9] and Aspergillus niger amine oxidase.[10] A preliminary report from our lab claimed inactivation of BPAO by **4** as well as by a diamine version **5** of 2-chloroallyl amine.[11]



An early study reported that 1,4-diamino-2-*butene* (without specification of geometrical isomerism), was converted to pyrrole by pig kidney diamine oxidase (PKDAO).[12] As a follow-up to this report, we evaluated the individual *trans*- (**6**) and *cis*-1,4-diamino-2-butene (**7**) isomers with PKDAO.[13] As shown in Scheme 2, both isomers were found to be substrates and irreversible mechanism-based inactivators of DAO. The *trans* isomer **6** was the more potent inactivator, exerting almost complete inhibition at 1 mM in 1 h, whereas the *cis* isomer **7** exhibited only 54% inhibition under the same conditions, owing to competitive ring closure of the initial *cis*-aminoenal turnover product. The independently synthesized *trans*-aminoenal was found to be a potent enzyme inactivator.[13] In addition it was shown that pyrrole formation starting with the *trans* isomer **6** arises from addition–elimination of substrate or product amine to the *trans*-aminoenal turnover product, converting it to the *cis*-aminoenal.[13] Interestingly, both **6** and **7** appear to be pure substrates for the grass pea diamine oxidase.[14] The lack of inactivation in these cases suggests the absence of an active-site nucleophile suitably positioned to be irreversibly alkylated by the immediate aminoenal turnover products.



Scheme 2.

The preliminary reports that **4**[8,11] and **5**[11] are mechanism-based inactivators of BPAO raises the question as to whether this enzyme manifests some selectivity for diamines over monoamines, at least in terms of substrate action leading to inactivation. Thus, to clarify if diamine-based inactivators could be relied upon to exert selective inactivation of DAO, we investigated the structural dependence of diamine-based inactivation on BPAO by varying the nature of the second amino group in compounds **4**–**7**.

Results

Design and synthesis

The chosen structural modifications were to convert the second primary amine group to a secondary or tertiary amino group or to acylate it. Alkylation would prevent metabolism at that end (*N*-methyl secondary amines are not substrates for the copper amine oxidases) but not affect the charge properties. On the other hand, acylation would abolish metabolism at that end as well as prevent protonation. In the case of the unsymmetrical compound **5**, there would be two different regioisomers possible for each type of modification. According to these guidelines, the compounds in addition to **4**–**7** that were prepared here for study are given in Chart 1.

trans- (**6**) and *cis*-1,4-Diamino-2-butene (**7**) were prepared as previously reported.[13] Though **4** has been made by the Gabriel synthesis from 1,4-dichloro-2-butyne, we found it more convenient to convert the dichloride directly to **4** with NH_4OH.[15] The secondary and tertiary amine analogues of **4** were synthesized according to the routes shown in Scheme 3. Monosubstitution of 1,4-dichloro-2-butyne with potassium phthalimide to give 1-chloro-4-phthalimido-2-butyne (**19**)[16] took advantage of the use of excess dichloride to prevent bis-derivatization (unreacted dichloride was readily separated). When the primary amines methylamine, benzylamine, and phenethylamine were added in excess to **19**, the secondary amine analogues **8**–**10** were afforded directly by a one-pot reaction, with removal of the phthaloyl protecting group occurring under the basic reaction conditions. However, when the secondary

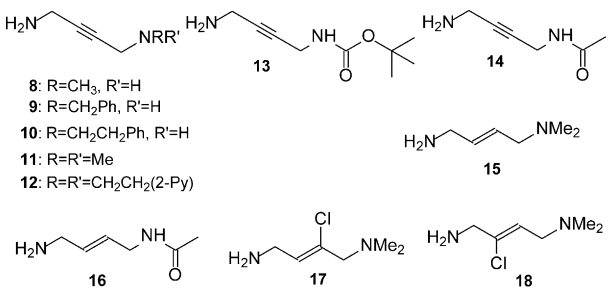
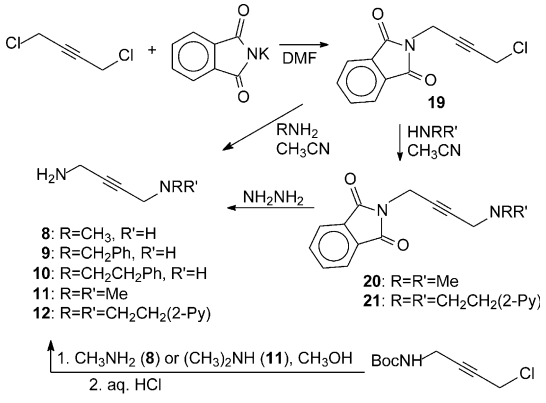


Chart 1. The compounds prepared for evaluation of inhibition of bovine plasma amine oxidase.

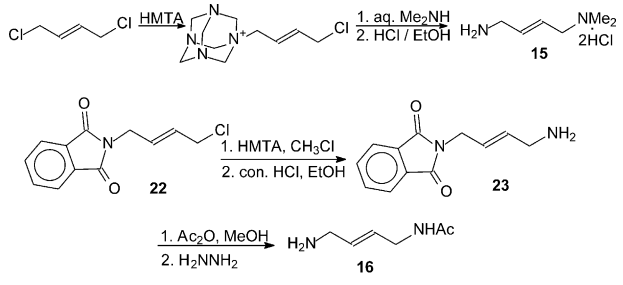
amines dimethylamine and bis[2-(2-pyridyl)ethyl]amine were used, the intermediates **20** and **21** were first obtained, requiring deprotection with hydrazine to give desired tertiary amines **11** and **12** (Scheme 3). In later studies, it was found that superior yields of the *N*-methyl and *N*,*N*-dimethyl analogues **8** and **11** could be obtained from the reaction of Boc-protected 4-chloro-2-butynamine[17] with methanolic methylamine or dimethylamine, respectively, followed by deprotection (Scheme 3). Monoacylation of **4** with di-*tert*-butyl dicarbonate at pH 10 gave *N*-Boc-1,4-diamino-2-butyne (**13**). Acetylation of the latter compound (Ac_2O, Et_3N) followed by acid hydrolysis gave *N*-acetyl-1,4-diamino-2-butyne (**14**).

trans-*N*,*N*-Dimethyl-1,4-diamino-2-butene (**15**) was prepared starting with monosubstitution of *trans*-1,4-dichloro-2-butene with hexamethylenetetramine (HMTA) on one side, followed by substitution with dimethylamine on the other side, and finally acid hydrolysis as shown in Scheme 4. *trans*-*N*-Acetyl-1,4-diamino-2-butene (**16**) was prepared from *trans*-1,4-dichloro-2-butene by its reaction with potassium phthalimide to give **22**, which was in turn reacted with HMTA followed by acid hydrolysis to give **23**, followed by acetylation and finally hydrazinolysis of the phthalimide protecting group (Scheme 4).

Z-2-Chloro-1,4-diamino-2-butene (**5**) was prepared by addition of HCl to 1,4-diamino-2-butyne (**4**) as described.[18] *Z*-1-Amino-3-chloro-4-(dimethylamino)-2-butene (**17**) and *Z*-1-amino-2-chloro-4-(dimethylamino)-2-butene (**18**) were prepared as a mixture by the same procedure[18] from 1-amino-4-(dimethylamino)-2-butyne (**11**). Since it was very difficult to separate **17** and **18** directly, we added di-*tert*-butyl dicarbonate to the mixture to give Boc protected compounds **24** and **25**, which could be separated by flash chromatography and deprotected by acid hydrolysis to give the individual isomers **17** and **18** (Scheme 5).



Scheme 3.

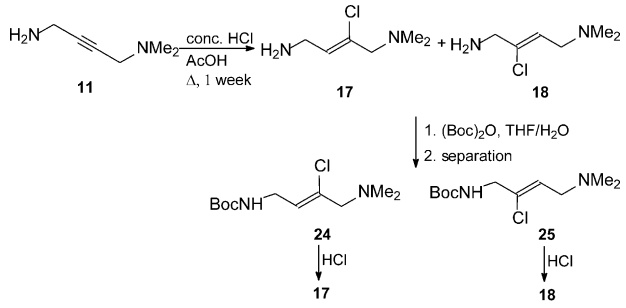


Scheme 4.

Structural distinction between **17** and **18** was aided by comparison of their ^1H and ^{13}C NMR spectra to that of **5**. As shown in Chart 2, the ^1H NMR (3.89 ppm) and ^{13}C NMR (37.2 ppm) signals of C4 in **5** match those of C1 in **17** but with no position in **18**. Also, the ^1H NMR (4.01 ppm) and ^{13}C NMR (45.4 ppm) signals of C1 in **5** are consistent with those of C1 in **18** but with no position of **17**. The stereochemistry in **17** and **18** was assigned as *Z*, as in the case of **5**, based on the knowledge that HCl addition to alkynes in HOAc occurs *anti* rather than *syn*.[19] In the case of **17**, we confirmed the stereochemistry through an NOE difference experiment. Enhancements of greater than 0.3% that were observed for irradiation of the two methylene and vinyl positions are shown in Chart 3. The enhancements observed between the two methylene positions were only 0.1 and 0.2%, as opposed to large values that would be expected in the case of the *E* isomer.

Enzyme studies

All compounds were evaluated for their ability to inactivate the benzylamine oxidase activity of commercially



Scheme 5.

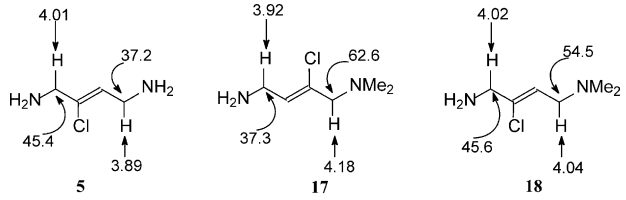


Chart 2. Chemical shift correlation of compounds **5**, **17**, and **18**.

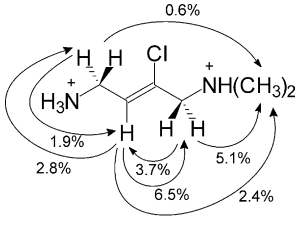


Chart 3. Nuclear Overhauser effect enhancements for compound **17** in CD_3OD.

available BPAO (Sigma) at 30 °C, pH 7.2, determined by assaying the rate of benzaldehyde production, and the data are listed in Table 1. Irreversibility of inhibition was demonstrated by comparing the activity before and after gel filtration to remove non-covalently bound small molecules and additionally in the cases of **8** and **11** by 24 h dialysis against 100 mM pH 7.2 phosphate buffer. For most inhibitors, the time-dependent loss of enzyme activity was incomplete and plateaued at an activity level that monotonically decreased as the initial concentration of inhibitor increased. This plateau behavior is indicative of classical partitioning between turnover and inactivation and permits determination of partition ratios.[20]

Table 1. Inhibitory data of compounds

Compd	IC_{50} (mM)[a]	Partition ratio	Kinetic data
4	0.020 (0.012)[d]	20	$K_I = 0.92$ mM, $k_{inact} = 1.3$ min^{-1}
5	0.024	29	$K_I = 0.19$ mM, $k_{inact} = 1.7$ min^{-1}
6	2	No[b]	$K_I = 11.8$ mM, $k_{inact} = 0.14$ min^{-1}
7	Reversible		73% inhibition in 1 min, 2.5 mM[c]
8	0.4[d]	1800[d]	60% inhibition in 26 min, 2 mM
9	1.0	555	
10	0.5	330	
11	1.0 (1.4)[d]	4000[d]	50% inhibition in 50 min, 2.5 mM
12	0.1	138	60% inhibition in 25 min, 0.15 mM
13	1.2[e]	e	
14	0.35[e]	e	
15	3.5	N.D.	
16	4[e]	e	
17	2.0	N.D.	53% inhibition in 30 min, 2 mM
18			20% inhibition at 2 mM[f]

ND, not determined.
[a]The number listed represents the concentration needed to achieve 50% inactivation at the point of activity plateau (5–120 min) for those inhibitors that exhibited partitioning (partition ratio listed), or the concentration needed to achieve 50% inactivation in 30 min, for those inhibitors that did not exhibit plateau behavior. Unless indicated otherwise, the loss of activity is irreversible even after gel filtration and/or 22-h dialysis.
[b]No evidence of plateau behavior was seen for the concentration range used.
[c]The activity of enzyme inhibited by **7** recovered to 75% over 9 min.
[d]This measurement used BPAO obtained from Worthington.
[e]The enzyme inhibited by these compounds lost activity within 30 min and then began to recover. The activity recovered to ~90% in ≤12 h.
[f]The inhibition was not time-dependent.

1,4-Diamino-2-butyne-based inactivators

Initially the inactivation of BPAO by 1,4-diamino-2-butyne (**4**)[8,11] was reinvestigated, since kinetic parameters and the partition ratio were not reported. In the concentration range of 40–400 μM, loss of enzyme activity followed a simple exponential as shown in Figure 1. K_I and k_{inact} values (Table 1) determined by the method of Kitz and Wilson[21] (Fig. 2) indicate that **4** is a very efficient inactivator of BPAO ($k_{inact}/K_I = 1.4$ mM^{-1} min^{-1}). Although complete inactivation by **4** was observed at 0.1 mM and higher, loss of activity at lower concentrations reached a plateau, indicative of a partitioning between inactivation and turnover. The concentration of **4** needed to reach a plateau with 50% loss of activity was 20 μM. From the plateau data (not shown), the graphically-determined partition ratio was found to be 20. BPAO inactivated by **4** did not recover activity either by gel filtration or by overnight dialysis. Evidence for modification of the active site was that treatment of the inactivated enzyme with phenylhydrazine did not result in the characteristic absorption at 450 nm resulting from derivatization of the quinone cofactor.[22] In addition, samples of the enzyme that were 95–100% inactivated by **4** exhibited only 10–20% of the nitroblue tetrazolium (NBT) quinone redox cycling activity[23] of the unmodified enzyme, following denaturation.

The mono *N*-methyl- (Fig. 3), *N*-benzyl-, and *N*-phenethyl- secondary amine analogues **8**–**10** of 1,4-diamino-2-butyne exhibited a markedly reduced inhibitory potency compared to **4**. Nonetheless, all three exhibited a concentration- and time-dependent irreversible inactivation characterized by plateau behavior indicative of partitioning (plateau-determined IC_{50} values and partition ratio data is given in Table 1). The *N*,*N*-dimethyl derivative **11** exhibited an even weaker inhibitory potency than the mono *N*-methyl derivative **8** but was

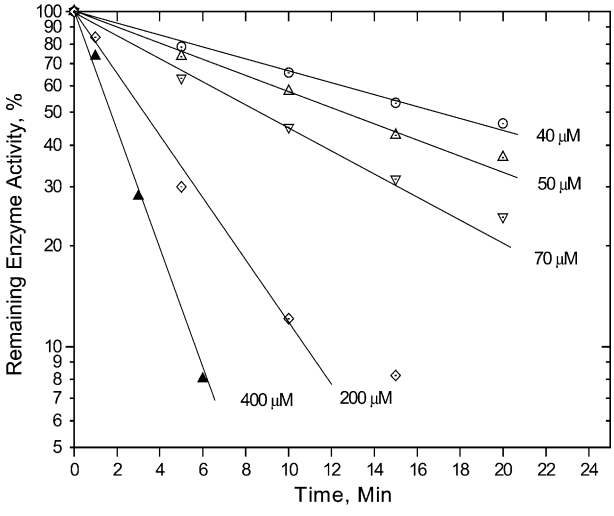


Figure 1. Time dependent inactivation of BPAO by various concentrations of 1,4-diamino-2-butyne (**4**).

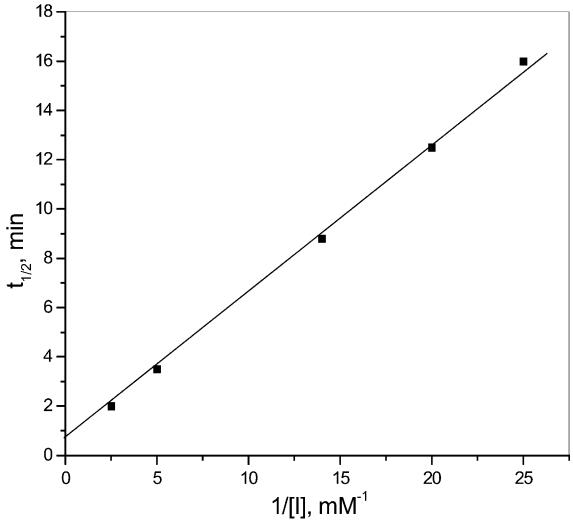


Figure 2. Kitz and Wilson plot for inactivation of BPAO by **4**.

again an irreversible inactivator. For both **8** and **11**, further loss of enzyme activity after about 2 h was very slow but still apparent (see Fig. 3 for **8**). The lack of a clear plateau at the point of consumption of inhibitor has been seen before, and was interpreted to reflect a slow inhibition by the accumulated aldehyde turnover product.[24] The listed plateau IC_{50} values and partition ratios (Table 1) were determined in these two cases from the psuedo-plateau points of the observed biphasic behavior. Whereas BPAO inactivated by **8** and **11** failed to react with phenylhydrazine, preparations of BPAO inactivated by **8** (95%) or **11** (94%) still gave 40–50% of the NBT redox cycling response of the unmodified enzyme.

The huge drop in inactivation potency seen upon addition of even a single *N*-methyl group to diamine **4** is remarkable, and the large partition ratio seen for this compound (**8**) suggests that it is still being efficiently processed by BPAO, but mainly as a substrate rather than inactivator. To confirm the substrate activity of both **8** and **11**, O_2 uptake studies were performed. Dioxygen consumption was linear with concentration at low concentrations of inhibitor, but attempts to observe saturation behavior at higher concentration (to permit calculation of V_{max} and K_m) were thwarted by enzyme inactivation occurring within the (short) time frame of determining the O_2 consumption rate. In fact, the measured O_2 consumption actually began to decrease with increasing concentration or **8** and **11** above about 2–3 mM. Thus, for comparative purposes, the second-order rate constant was computed from the linear portion of the plot of O_2 consumption rate versus concentration at low concentration. Under the conditions of the assay (pH 7.2, 30 °C), the *k* values for **8**, **11**, and benzylamine were 8.6, 9.3, and 22 mM^{-1} min^{-1}, respectively, indicating that **8** and **11** are excellent substrates for BPAO, though not as good as benzylamine. Although a determination of substrate behavior for the parent diamine **4** was precluded by the potent inactivation that occurs even at relatively low concentrations, one can compare the above numbers to the calculated pseudo-second-order processing of **4** as an inactivator ($k_{inact}/K_I = 1.4$ mM^{-1} min^{-1}).

Interestingly, another tertiary amine analogue, *N*,*N*-[bis(2-pyridyl)ethyl]-1,4-diamino-2-butyne (**12**) exhibited a reasonably potent irreversible inactivation (plateau IC_{50} value of 0.1 mM). The relatively slow onset of inhibition even though [I]≫[E] suggests that this compound is not inhibiting by chelating the Cu(II) out of the enzyme. The mono-acetyl and mono-Boc derivatives **14** and **13** exhibited an 18- and 60-fold drop, respectively, in BPAO inhibitory potency relative to **4** (Table 1), and in these cases, the partially inhibited enzyme recovered slowly over time (see Fig. 4 for **14**). The lack of permanent inhibition in these cases may reflect an instability (e.g., toward hydrolysis) of the covalent adduct generated.

Allylamine-based inhibitors

Although it has been reported that allylamine is a substrate rather than inactivator of BPAO,[7] the fact that the allylic *diamines* **6** and **7** inactivate PKDAO[13] suggested that they may also inactivate BPAO. *trans*-1,4-Diamino-2-butene (**6**) was found to be a time- and concentration-dependent but relatively weak irreversible inactivator of BPAO, inducing a 50% enzyme activity loss in 30 min at 2 mM. The graphically determined K_I and k_{inact} values (Table 1) indicate an inactivation efficiency (k_{inact}/K_I) for **6** that is 119 times lower than its acetylenic analogue **4**. The *N*,*N*-dimethyl derivative **15** of **6** was an even less potent inactivator, but was still irreversible (Table 1). On the other hand, whereas *trans*-*N*-acetyl-1,4-diamino-2-butene (**16**) also exhibited weaker inhibition than **6** (Table 1), enzyme activity recovered slowly in this case, as was observed for the mono-acyl derivatives **13** and **14** of 1,4-diamino-2-butyne. On account of the weak inhibitory properties of **15** and **16**, we did not pursue the determination of inhibitory kinetic parameters.

cis-1,4-Diamino-2-butene (**7**) exerted 73% inhibition at 1 min at 2.5 mM, with no clear time-dependent activity loss, but the enzyme activity recovered very rapidly (Fig. 4). The transient inhibition of benzylamine

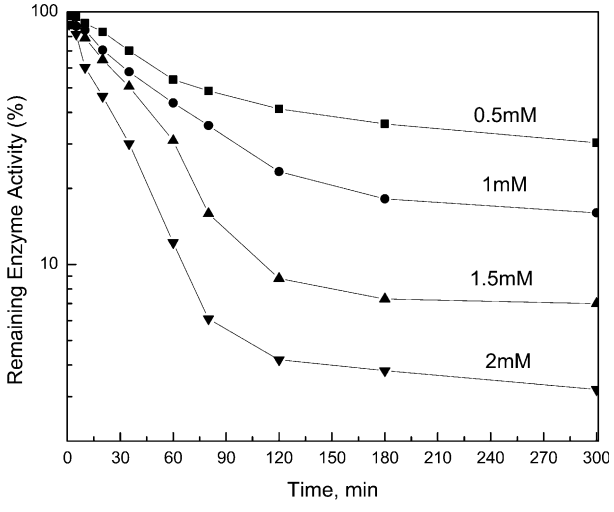


Figure 3. Time dependent inactivation of BPAO by various concentrations of **8**.

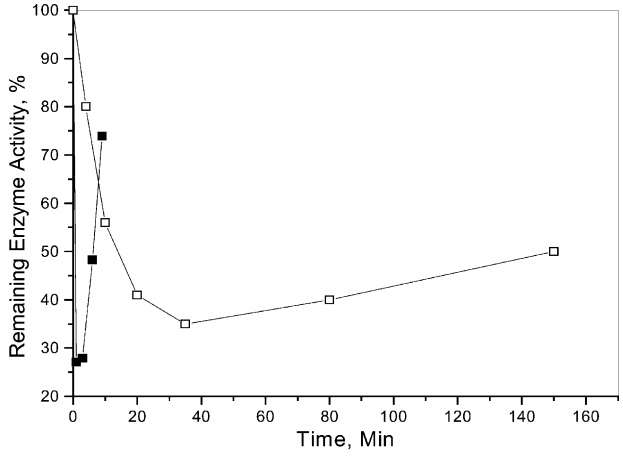


Figure 4. Effect of incubation time on inactivation of BPAO by *cis*-1,4-diamino-2-butene (**7**, ■) and *N*-acetyl-1,4-diamino-2-butyne (**14**, □).

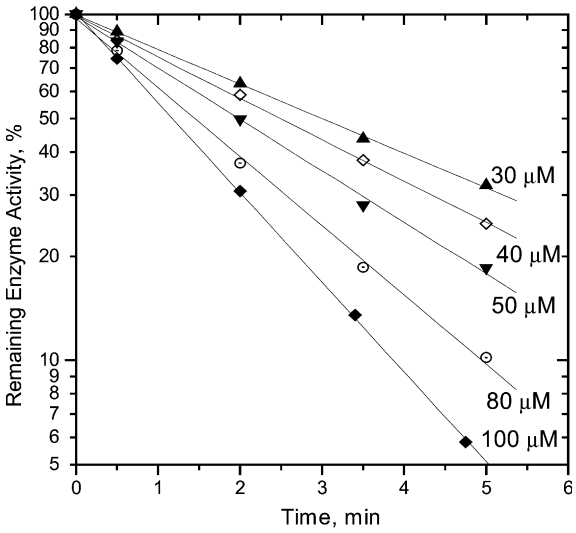


Figure 5. Effect of incubation time on inactivation of BPAO by various concentrations of **5**.

oxidation by **7** is most likely simply the result of substrate activity of **7** (it must bind more tightly than benzylamine) until it is consumed. The rapid formation of pyrrole from the *cis*-aminoenal turnover product (Scheme 2) would protect the enzyme from being alkylated, as was deduced for DAO,[12,13] thus rationalizing the lack of irreversible enzyme inactivation in this case.

Chloroallylamine-based inactivators

Z-2-Chloro-1,4-diamino-2-butene (**5**) was found to be a potent time- and concentration-dependent irreversible inactivator of BPAO (Fig. 5), consistent with preliminary reports.[11,25] Strict pseudo-first-order behavior permitted Kitz and Wilson determination of kinetic parameters (Table 1) that indicate this compound is the most efficient (k_{inact}/K_I) inactivator of BPAO studied here, about 6 times more so than 1,4-diamino-2-butyne (**4**). Nonetheless, at low concentration, the activity versus time plots reached plateaus, indicative of partitioning, permitting graphical determination of a partition ratio of 29. It is noteworthy that the behavior of **5** is so similar to that of **4**, suggesting that these two closely related compounds, differing by the elements of HCl, may inactivate BPAO via a common intermediate. Consistent with this possibility, a preparation of BPAO that was 99% inhibited by **5** exhibited only 5–10% of the NBT redox cycling capacity of the control preparation, similar to what was observed for **4**.

The two *N*,*N*-dimethyl regioisomers of **5** were designed to evaluate how the positional dependence of the chlorine atom with respect to the primary amine moiety influences inactivation potency. *Z*-1-Amino-3-chloro-4-(dimethylamino)-2-butene (**17**), which contains a 3-chloroallylamine part-structure, exhibited a time-dependent irreversible inactivation of BPAO, but with a weak inhibitory potency. Curiously, the 2-chloro regioisomer **18**, that contains the 2-chloroallylamine part-structure, did not exhibit time-dependent inactivation, and exerted only 20% inhibition at 2 mM.

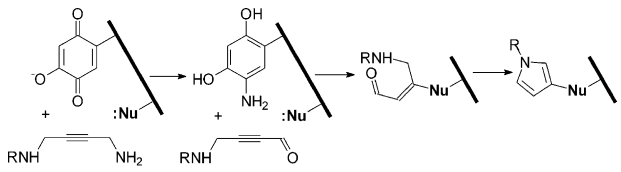
Discussion

1,4-Diamino-2-butyne (**4**) has been confirmed to be a potent mechanism-based inactivator of BPAO, as it is of diamine oxidases. In fact, although inactivation of plant DAO by **4** is not so pronounced so as to preclude measurement of its substrate kinetic parameters,[26] **4** appears to be a more pure inactivator of BPAO. Its increased potency relative to propargylamine (**1**) as well as the ability of *trans*-1,4-diamino-2-butene (**6**) but not allylamine (**3**) to inactivate BPAO, suggests that the second amino group in the diamine inhibitors provides for some type of special recognition, either in terms of binding or in terms of the inactivation chemistry. Due to structural similarity of diamine oxidases with the other copper amine oxidases, the improved inactivating potency of **4** and **6** relative to **1** and **3** for BPAO may reflect a common recognition feature for both enzymes, even though diamines are not known to be especially good substrates of BPAO. This latter point probably deserves careful scrutiny.

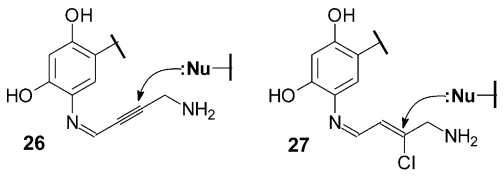
If the improved inhibitory efficacy of **4** and **6** is due to a facilitation of binding by the second positive charge, then one would expect that at least small *N*-alkyl and *N*,*N*-dialkyl secondary and tertiary amine derivatives would preserve inactivating potential, whereas the mono *N*-acyl derivatives might not. The *N*-acyl derivatives **13** and **14** of **4** were found to be weak and at least partially reversible inhibitors of BPAO, consistent with the requirement of at least a second basic center on the installation of a permanent covalent mode of inactivation. However, even mono-*N*-methylation of **4** resulted in a marked weakening of inhibitory potency. The finding that inactivation of BPAO by **4** but not by **8** results in loss of redox cycling integrity of the quinone cofactor, suggests that **4** induces a very special type of covalent inactivation that incorporates a modified quinone cofactor alone or together with an active site residue. The distinctive inactivation by **4** must reflect a mechanism either that requires both primary amino termini or that is exquisitely sensitive to even the subtle steric and/or pK_a change accompanying introduction of a single methyl group.

Because plasma amine oxidase is usually considered to have a high affinity for arylalkylamines, we investigated whether the addition of a benzyl (**9**) or phenethyl group (**10**) to one amino terminus of 1,4-diamino-2-butyne might improve inactivation efficiency for BPAO. Both compounds had reduced inactivating potency relative to **4**, and they were not improved inhibitors relative to the *N*-methyl compound **8**.

Frebort and coworkers[26] recently proposed a mechanism for the inactivation of plant amine oxidases by **4** that involves its metabolism to 4-amino-2-butynal (or its tautomer 4-amino-1,2-butadienal), which is a potent Michael acceptor and alkylates the enzyme to give a residue-bound 4-amino-2-butenal moiety that cyclizes to a pyrrole (Scheme 6, R = H). Pyrrole formation would not be possible with either the *N*,*N*-dimethyl or *N*-acyl analogues, though the mono-*N*-alkyl analogues



Scheme 6.



Scheme 7.

would potentially be able to follow this same mechanism, giving *N*-alkylpyrroles (Scheme 6, R = alkyl). Although the fates of *N*-methyl compound **8** and *N*,*N*-dimethyl compound **11** with the plant enzyme have not been investigated, the data obtained with BPAO are inconsistent with operation of a common mechanism. First, inactivation of BPAO by **4** was accompanied by all but 10–20% loss of the NBT redox cycling activity, suggesting that the inactivated enzyme in this case contains a modified cofactor, though side-chain modification in addition cannot be excluded. Second, *both* the *N*-methyl and *N*,*N*-dimethyl compounds were considerably weaker inhibitors, each exhibiting only about 50% loss of NBT signal. The implications of partial loss of redox-cycling are unclear, possibly reflecting a modified cofactor with reduced but still active redox properties, but more likely reflecting a heterogeneous population of inactivated enzyme, with and without modified redox-inactive cofactor. If the mechanism of Scheme 6 were operative for BPAO, the monomethyl inhibitor **8** should have behaved more like **4** than like **11**. Further studies will be needed to elucidate the basis of the uniquely potent inhibition of BPAO effected by **4**.

Despite *trans*-1,4-diamino-2-butene (**6**) being a relatively weak inactivator relative to **4**, *N*,*N*-dimethylation at one end of **6** significantly diminished its inactivation potency, as in the case of **4**, again pointing to some special role of the second primary amine group. The *cis*-allyldiamine analogue **7** behaved as a substrate rather than as an inactivator, apparently because the immediate *cis* aminoenal product cyclizes to pyrrole as turnover product before alkylation occurs (Scheme 2).

Abeles had shown many years ago that 2-chloroallylamine has similar potency to propargylamine as an inactivator of BPAO.[7] It is thus important to note that upon extension of these two compounds to 1,4-diamines, *Z*-2-chloro-1,4-diamino-2-butene (**5**) and 1,4-diamino-2-butyne (**4**) are now seen to have very similar and more potent inactivating potential. As mentioned before, this parallel behavior may indicate a common reactive intermediate mechanism of inactivation since the two compounds differ only by the elements of HCl. One possibility would be that after processing of **4** and **5** to the product Schiff base stages **26** and **27** (Scheme 7), addition of an active site nucleophile to **26** and substitution of the same active site nucleophile for Cl^- (addition-elimination) in **27** would give the same initial product. Alternatively, tautomerization of **26** and elimination of HCl from **27** would generate the identical allenylimine, which could be the alkylating moiety.

Importantly, in our efforts to determine the effect of the two possible *N*,*N*-dimethylation regiochemistries on the chloroallyldiamine **5**, the isomer that contains the 2-chloroallylamine part-structure (**18**) was found to be a weaker inactivator than the isomer (**17**) that contains the 3-chloroallylamine part-structure (though both were relatively weak). The greater potency of **17** is consistent with the finding that the *E* and *Z* isomers of 3-chloroallylamine were much more potent inhibitors of BPAO than 2-chloroallylamine,[27] consistent with the addition-elimination mechanism depicted in Scheme 7. Overall, however, although possibly the first step, simple conjugate addition would not explain why, for example, inactivation by the *N*-acyl inhibitors **13** and **14** is reversible. The maturation of the initial alkylated enzyme nucleophiles must vary as a function of the status of the second amino group according to multistep mechanisms such as shown in Scheme 6.

Conclusions

The results described here extend previous reports on the inactivation of BPAO by amines bearing β-unsaturation.[7,8,11] It is evident that propargylic and chloroallylic diamines are highly potent inhibitors, more so than the simple allylic diamines, and that the inactivators bearing two primary amine termini (**4**–**6**) are more potent inhibitors than the corresponding derivatives where one of the primary amino termini is converted to a secondary or tertiary amine, or to an amide group. Though of variable potency, all of the diamines were irreversible inactivators, distinguishing them from the *N*-acyl analogues, which displayed weak inhibition, and in each case the partially inhibited enzyme recovered activity over time. Though the mechanisms responsible for enzyme inactivation have not been elucidated, multiple mechanisms must be at play for the different families of compounds, and cofactor modification appears to occur alternatively or additionally to alkylation of an active site residue in the case of the uniquely potent inhibitors **4** and **5**. The finding that **4**, previously known to be a potent inhibitor of diamine oxidases, is also a potent inactivator of BPAO, is suggestive of unexpected similarities between these two enzymes, and thus it is probably unwise to try to inactivate diamine oxidase selectively with diamine-based inhibitors.

Experimental

General methods

^{1}H NMR spectra were obtained on a Varian Gemini 300 (^{13}C NMR at 75 MHz) or 200 (^{13}C NMR at 50 MHz) instruments, with chemical shifts being referenced to TMS or the solvent peak. In the ^{13}C NMR line listings, attached proton test (APT) designations are given as (+) or (−) following the chemical shift. The NOE difference spectra were recorded using a Varian Inova 600. High-resolution mass spectra (HRMS, electron impact) were obtained at 20–40 eV on a Kratos MS-25A instrument. UV–vis spectra were obtained using a jacketed (temperature-controlled) cell compartment and Perkin-Elmer PECSS software. Doubly distilled water was used for all enzyme experiments and model studies. Melting points are uncorrected. Thin-layer and preparative thin-layer chromatography were run on Merck silica gel 60 plates with 254 nm indicator. All column chromatography was run using flash-grade silica gel. All solvents, reagents, and organic fine chemicals were the most pure available from commercial sources. Bovine plasma amine oxidase (BPAO) (100 units/gram of protein) and PDX G. F. 25 were purchased from Sigma. In later studies, BPAO was purchased from Worthington Biochemical. All evaporations were conducted at reduced pressure using a rotary evaporator.

1,4-Diamino-2-butyne (4). A solution of 2.6 g (20 mmol) of 1,4-dichloro-2-butyne and 50 mL of concentrated NH_4OH was heated to 40 °C for 3 h in a 350 mL pressure bottle. The mixture rapidly became homogenous. Excess ammonia was evaporated carefully at room temperature, the remaining solution was acidified to pH 5, and the acidic solution was evaporated almost to dryness. The residue was adjusted to pH 11 and extracted with diethyl ether using a continuous extractor. The organic layer was dried (Na_2SO_4) and concentrated to afford a brown oil which was distilled to give 0.7 g (40%) of the main fraction of **4**[15] (16 mm Hg, 96 °C): mp 42–46 °C.

***Z*-2-Chloro-1,4-diamino-2-butene dihydrochloride (5).** A solution of 1,4-diamino-2-butyne dihydrochloride (350 mg, 2.23 mmol) was heated at reflux in a 1:1 mixture of concd HCl and glacial acetic acid (30 mL) for 1 week. The reaction mixture was concentrated and the solid was recrystallized from glacial AcOH to give the product **5·2HCl** in 72% yield: ^{1}H NMR (D_2O) δ 3.89 (d, J = 6.6 Hz, 2H), 4.01 (s, 2H), 6.28 (t, J = 6.6 Hz, 1H); ^{13}C NMR (D_2O) δ 37.2, 45.4, 124.5, 131.7.

1-Chloro-4-phthalimido-2-butyne (19). 1,4-Dichloro-2-butyne (20 mL, 0.204 mol) was added to the stirred suspension of potassium phthalimide (4.7 g, 25.5 mmol) in DMF (30 mL). The reaction mixture was heated to 100 °C for 2 h. After cooling, 50 mL of water was added and the precipitated solid was filtered and dried at high vacuum to give 1-chloro-4-phthalimido-2-butyne as a white solid in 70% yield: ^{1}H NMR ($CDCl_3$) δ 4.09 (t, J = 2.13 Hz, 2H), 4.48 (t, J = 1.71 Hz, 2H), 7.72–7.81 (4H). ^{13}C NMR ($CDCl_3$) δ 27.3, 30.2, 77.9, 80.0, 123.6 (2C), 132.0 (2C), 134.3 (2C), 167.0 (2C).

***N*-Methyl-1,4,-diamino-2-butyne diperchlorate (8) from 19.** Methylamine gas was slowly bubbled through a solution of 2.3 g (10 mmol) of **19** in 50 mL of acetonitrile at room temperature for 40 h. The solvent and excess methylamine was removed by evaporation to afford 1.9 g (87%) of 1-phthalimido-4-(methylamino)-2-butyne as a white solid. A solution of the latter (1.3 g, 6 mmol) and hydrazine (85%, 15 mL) in 50 mL of ethanol was heated at reflux for 2.5 h. After cooling the mixture to 0 °C, phthalhydrazide was removed by filtration. Evaporation of the filtrate left the crude amine free base which was subjected to flash column chromatography (CH_2Cl_2–MeOH–NH_4OH 10:10:1 as eluent) to afford 0.44 g (75%) of **8**. The free base form is highly hygroscopic and was converted to the di-$HClO_4$ salt immediately: ^{1}H NMR (CD_3OD) δ 2.82 (s, 3H), 3.95 (t, J = 1.82 Hz, 2H), 4.04 (t, J = 1.84 Hz, 2H); ^{13}C NMR (CD_3OD) 30.3 (+), 33.1 (−), 38.9 (+), 78.2 (+), 81.8 (+).

***N*-Benzyl-1,4-diamino-2-butyne dihydrochloride (9).** A solution of 3.0 g (12.8 mmol) of **19** and 8.3 g (76.8 mmol) of benzylamine in dry CH_3CN (50 mL) was heated at reflux for 6 h under Ar. The solid generated during the reaction was filtered, and the filtrate was concentrated in vacuo. The residue obtained upon solvent evaporation was subjected to column chromatography using MeOH as eluent to give the free base **9** as an oil in 39% yield: ^{1}H NMR ($CDCl_3$) δ 2.74 (s, 3H), 3.30 (s, 2H), 3.37 (s, 2H), 3.74 (s, 2H), 7.14–7.26 (5H); ^{13}C NMR ($CDCl_3$) δ 31.18, 37.57, 52.37, 80.77, 83.24, 127.13, 128.38 (appears to be a composite of two 2C signals), 139.30. The free base (0.8 g) was dissolved in CH_2Cl_2 (10 mL) and ethyl ether (10 mL), and HCl gas was bubbled through the solution for 10 min. The generated solid was filtered and recrystallized from EtOH containing a trace of H_2O to give **9·2HCl** in 92% yield: mp 215–217 °C. ^{1}H NMR (D_2O) δ 3.96 (s, 2H), 4.01 (s, 2H), 4.35 (s, 2H), 7.51 (s, 5H); ^{13}C NMR (D_2O) δ 29.13, 35.69, 50.21, 76.85, 80.26, 129.46 (2C), 130.03 (appears to be a composite of two (C + 2C) signals), 130.32. Anal. calcd for $C_{11}H_{16}Cl_2N_2·0.8H_2O$: C, 50.51; H, 6.78; N, 10.71. Found: C, 50.23; H, 6.25; N, 10.88. HRMS calcd for $C_{11}H_{14}N_2$ ($M^+ - H$) m/z (rel intensity) 173.1079, found 173.1075 (9%).

***N*-Phenethyl-1,4-diamino-2-butyne dihydrochloride (10).** A solution of 2.0 g (8.6 mmol) of **19** and 8.3 g (68.5 mmol) of phenethylamine in dry CH_3CN (40 mL) was heated at reflux for 12 h under Ar. The solid which was generated during the reaction was filtered, and the filtrate was concentrated in vacuo to remove CH_3CN and excess phenethylamine. The residue was subjected to column chromatography using MeOH as eluent to give the free base **10** in 41% yield: ^{1}H NMR ($CDCl_3$) δ 1.50 (br s, 3H), 2.83 (t, 2H), 2.93 (t, 2H), 3.43 (s, 4H), 7.22–7.31 (5H). ^{13}C NMR ($CDCl_3$) δ 31.42, 36.07, 38.37, 49.91, 80.30, 83.82, 126.17, 128.44 (2C), 128.66 (2C), 139.78. The free base (0.6 g) was dissolved in CH_2Cl_2 (10 mL) and ether (10 mL), and HCl gas was bubbled through the solution for 10 min. The generated solid was filtered and recrystallized from EtOH to give **10·2HCl** in 90% yield: mp 221.0–222.5 °C. ^{1}H NMR (D_2O) δ 3.07 (t, 2H), 3.46 (t, 2H), 3.93 (s, 2H), 4.01 (s,

2H), 7.35–7.44 (5H). ^{13}C NMR (D_2O) δ 29.12, 31.67, 36.60, 47.96, 76.77, 80.12, 127.63, 128.97 (2C), 129.27 (2C), 136.26. Anal. calcd for $C_{12}H_{18}Cl_2N_2$: C, 55.18; H, 6.95; N, 10.73. Found: C, 54.90; H, 6.87; N, 11.18.

***N*,*N*-Dimethyl-1,4-diamino-2-butyne dihydrochloride (11) from 19.** Triethylamine (7.2 mL, 51.6 mmol) was added to a mixture of **19** (4 g, 17.2 mmol) and dimethylamine hydrochloride (2.8 g, 34.4 mmol) in 60 mL of CH_2Cl_2. After stirring overnight at room temperature, the reaction mixture was extracted with cold water. The organic layer was dried over Na_2SO_4, concentrated, and subjected to flash column chromatography using EtOAc as eluent to give 1-(*N*,*N*-dimethyl)amino-4-phthalimido-2-butyne (**20**) as a solid in 85% yield: ^{1}H NMR ($CDCl_3$) δ 2.15 (s, 6H), 3.11 (t, $J=1.95$ Hz, 2H), 4.39 (t, $J=1.92$ Hz, 2H), 7.63–7.77 (4H). ^{13}C NMR ($CDCl_3$) δ 27.23, 44.11 (2C), 47.86, 78.38, 78.44, 123.41 (2C), 132.00 (2C), 134.10 (2C), 166.98 (2C). A mixture of **20** (2.5 g, 10.3 mmol) and hydrazine monohydrate (0.5 mL, 10.3 mmol) in ethanol (50 mL) was heated at reflux for 1 h. After cooling to 0 °C, the reaction mixture was filtered and the filtrate was carefully distilled to obtain the free amine **11** (1.2 mL), which was treated with 1 N HCl to give the salt form, **11·2HCl** in 73% yield: ^{1}H NMR (D_2O) δ 2.99 (s, 6H), 3.99 (t, 2H), 4.16 (t, $J=1.95$ Hz, 2H). ^{13}C NMR (D_2O) δ 29.3, 42.4 (2C), 47.0, 75.7, 81.9. HRMS calcd for $C_6H_{12}N_2$ (M^+) *m*/*z* (rel. intensity) 112.1001, found 112.0997 (13%).

***N*-Methyl-1,4,-diamino-2-butyne (8) and *N*,*N*-dimethyl-1,4-diamino-2-butyne (11) from 1-*tert*-butyloxycarbonylamino-4-chloro-2-butyne.** 1-Amino-4-chloro-2-butyne·HCl was prepared by HCl-mediated hydrolysis of the product of reaction of hexamethylenetetramine with 1,4-dichloro-2-butyne:[16] ^{1}H NMR (D_2O) δ 3.91 (t, $J=1.39$ Hz, 2H), 4.30 (t, $J=1.39$ Hz, 2H). The amine salt (10.5 g, 75 mmol) was dissolved in 250 mL of water-methanol (5:1), and 1.1 equivalents of di-*tert*-butyl dicarbonate was added fractionally at room temperature while maintaining the pH at 10.0 by dropwise addition of 3 N NaOH. At the end of reaction, 200 mL of water was added and the mixture was extracted with CH_2Cl_2 (3×100 mL). The combined organic layer was dried (Na_2SO_4) and evaporated, and the residue was subjected to flash column chromatography with hexanes–CH_2Cl_2 (2:1) as eluent to give 1-*tert*-butyloxycarbonylamino-4-chloro-2-butyne[17] in 90% yield: ^{1}H NMR ($CDCl_3$) δ 1.46 (s, 9H), 3.97 (dt, $J=2.08$, 5.4, 2H), 4.14 (t, $J=2.08$ Hz, 2H), 4.70 (br, 1H). To a 50-mL pressure bottle was added 102 mg (50 mmol) of the latter Boc-protected compound and 20 mL of a 2 M solution of either methylamine or dimethylamine in methanol containing a catalytic amount of KI, and the mixture was heated to 50 °C for 2 h. After cooling to room temperature, the mixture was adjusted to pH 3 with 3 N HCl and extracted with diethyl ether (discarded). The aqueous layer was adjusted to 11 and extracted with diethyl ether (3×30 mL). The combined organic layer was dried (Na_2SO_4) and evaporated to afford the Boc-protected derivatives of **8** and **11**. The Boc group was removed by 3 N HCl in ethanol at room temperature to afford the dihydrochlorides of **8** and **11** in 60% yield. **11·2HCl**: ^{1}H NMR (CD_3OD) δ 3.00 (s, 6H), 3.98 (t, $J=2.01$ Hz, 2H), 4.23 (t, $J=2.01$ Hz, 2H); ^{13}C NMR (CD_3OD) δ 30.19 (+), 42.94 (−), 47.59 (+), 76.99 (+), 83.57 (+).

***N*,*N*-Bis[2-(2-pyridyl)ethyl]-1,4-diamino-2-butyne tetrahydrochloride (12).** A solution of 1.55 g (6.6 mmol) of **19** and 3.04 g (13.4 mmol) of bis[2-(2-pyridyl)ethyl]amine in dry CH_3CN (40 mL) was heated at reflux for 8 h. The reaction mixture was concentrated, and the residue was partitioned between CH_2Cl_2 and water. The organic layer was separated, dried over Na_2SO_4, and concentrated. The residue was subjected to column chromatography using CH_2Cl_2–MeOH (5:1) as eluent to give 4-bis[2-(2-pyridyl)ethyl]amino-1-phthalimido-2-butyne (**21**) in 84% yield: ^{1}H NMR ($CDCl_3$) δ 2.90 (br s, 8H), 3.47 (t, 2H), 4.45 (t, 2H), 7.04–7.10 (4H), 7.49 (2H), 7.68–7.86 (4H), 8.44 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 27.39, 36.27 (2C), 42.45, 53.53 (2C), 78.39, 78.48, 121.10 (2C), 123.28 (2C), 123.50 (2C), 132.10 (2C), 134.13 (2C), 136.21 (2C), 149.08 (2C), 160 30 (2C), 167.08 (2C). A solution of 1.8 g (4.5 mmol) of **21** and anhydrous hydrazine 0.2 mL in MeOH (40 mL) was heated at reflux for 2 h. The reaction mixture was filtered and the filtrate was concentrated in vacuo. The residue was dissolved in CH_2Cl_2, and HCl gas was bubbled through the solution for 15 min. The generated salt was filtered and recrystallized from EtOH to give **12·4HCl** in 88% yield: mp 156–159 °C. ^{1}H NMR (D_2O) δ 3.61 (t, 4H), 3.85 (t, 4H), 4.01 (s, 2H), 4.35 (br s, 2H), 7.97–8.08 (4H), 8.58 (t, 2H), 8.73 (d, 2H). ^{13}C NMR (D_2O) δ 28.23 (2C), 29.30, 42.89, 51.55 (2C), 74.90, 82.82, 126.25 (2C), 127.80 (2C), 141.84 (2C), 147.61 (2C), 150.56 (2C). Anal. calcd for $C_{18}H_{26}Cl_4N_4·0.5H_2O$: C, 48.13; H, 6.01; N, 12.47. Found: C, 48.13; H, 6.13; N, 12.17.

***N*-*tert*-Butyloxycarbonyl-1,4-diamino-2-butyne (13).** To a solution of 1,4-diamino-2-butyne (1 g, 11.9 mmol) in 20 mL of methanol–water (9:1) was added a solution of di-*tert*-butyl dicarbonate in MeOH, while maintaining the solution at pH 10-10.5 by adding aqueous NaOH, until about 70% of the 1,4-diamino-2-butyne was consumed according to TLC analysis. The reaction mixture was concentrated and partitioned between CH_2Cl_2 and brine, and the organic layer was dried over Na_2SO_4, concentrated, and subjected to flash column chromatography using EtOAc and then MeOH as eluents to afford **13** as a white solid in 37% yield: ^{1}H NMR ($CDCl_3$) δ 1.36 (s, 9H), 3.33 (t, $J=1.97$ Hz, 2H), 3.82 (d, $J=4.95$ Hz, 2H), 5.12 (br s, NH). ^{13}C NMR ($CDCl_3$) δ 28.3 (3C), 30.5, 31.4, 78.6, 79.7, 83.6, 155.4. HRMS calcd for $C_9H_{17}N_2O_2$ (MH^+) *m*/*z* (rel. intensity) 185.1290, found 185.1288 (2%).

***N*-Acetyl-1,4-diamino-2-butyne hydrochloride (14).** To a stirred solution of **13** (650 mg, 3.53 mmol) in dichloromethane (15 mL) was added triethylamine (0.98 mL, 7.06 mmol) and acetic anhydride (0.66 mL, 7.06 mmol). After stirring overnight, the reaction mixture was concentrated, and the residue was partitioned between $CHCl_3$ and brine. The organic layer was dried, concentrated, and subjected to flash column chromatography using EtOAc

as eluent to give 670 mg (83%) of *N*-acetyl-*N'*-*tert*-butyloxycarbonyl-1,4-diamino-2-butyne. ^{1}H NMR ($CDCl_3$) δ 1.34 (s, 9H), 1.90 (s, 3H), 3.79 (m, 2H), 3.90 (m, 2H), 5.30 (br s, NH, 1H), 6.93 (br t, NH, 1H). ^{13}C NMR ($CDCl_3$) δ 22.8, 28.3 (3C), 29.2, 30.4, 78.7, 79.3, 79.8, 155.6, 170.4. The latter intermediate (350 mg, 1.55 mmol) was treated with 1 mL of concd HCl. After 5 min, the reddish homogeneous solution was concentrated, and the residue was recrystallized from EtOH–Et_2O to give **14·HCl** in 61% yield: ^{1}H NMR (D_2O) δ 2.01 (s, 3H), 3.83 (s, 2H), 3.98 (s, 2H). ^{13}C NMR (D_2O) δ 21.9, 29.0, 29.3, 73.7, 83.1, 174.2. HRMS calcd for $C_6H_9N_2O$ (M^+-H), $C_6H_{10}N_2O$ (M^+), and $C_6H_{11}N_2O$ (MH^+) *m*/*z* (rel. intensity) 125.0715, 126.0793, and 127.0871, found 125.0713 (1%), 126.0777 (<1%), and 127.0859 (1%), respectively.

***trans*-*N*,*N*-Dimethyl-1,4-diamino-2-butene dihydrochloride (15).** Hexamethylenetetramine (4.0 g, 28 mmol) in ethanol (80 mL) was added dropwise to *trans*-1,4-dichloro-2-butene (15 mL, 0.14 mol). The mixture was allowed to stir at room temperature overnight, and then evaporated to dryness. The residue was triturated with $CHCl_3$ (10 mL×2), and the solid was filtered and dried under vacuum to give the light yellow *N*-(*trans*-4-chloro-2-butenyl)hexamethylenetetraminium chloride (7.0 g) as the intermediate. To a solution of dimethylamine hydrochloride (8.2 g, 0.1 mol) and KOH (7.1 g, 0.12 mol) in 30 mL of water cooled in an ice bath was added dropwise a solution of the intermediate salt (6.6 g, 25 mmol) in 60 mL water. The mixture was stirred at room temperature for 4 h and concentrated. The residue was dissolved in cold ethanol, and KCl was filtered off. The filtrate was concentrated, and the residue was partitioned between water and $CHCl_3$. The aqueous layer was concentrated to dryness to give a brown viscous oil, which was heated at reflux in a mixture of ethanol (75 mL) and con. HCl (10 mL) for 2 h. On cooling of the reaction mixture, the precipitate was filtered off, and the filtrate was concentrated to give an oily residue, which was crystallized by treating with *i*-PrOH–CH_3CN to give 200 mg of the product **15·2HCl**: mp 158–160 °C. ^{1}H NMR (CD_3OD) δ 2.9 (s, 6H), 3.7 (d, 2H), 3.9 (d, 2H), 6.1 (m, 2H). ^{13}C NMR (CD_3OD) δ 41.5, 42.9, 59.2, 126.0, 134.2. HRMS calcd for $C_6H_{14}N_2$ *m*/*z* 114.1157, found 114.1159 (M^+, 69%).

***trans*-*N*-Acetyl-1,4-diamino-2-butene (16).** To a suspension of potassium phthalimide (8.9 g, 48 mmol) in 50 mL of DMF was added *trans*-1,4-dichloro-2-butene (15 mL, 144 mmol) at once. The mixture was heated to 100 °C for 1 h, cooled to room temperature, and poured into 200 mL of ice-water. The precipitate was filtered, washed with water, and dried in air to give 6.0 g of *trans*-1-chloro-4-phthalimido-2-butene as a colorless solid: ^{1}H NMR ($CDCl_3$) δ 4.0 (d, 2H), 4.3 (d, 2H), 5.9 (m, 2H), 7.7 (q, 2H), 7.8 (q, 2H). A mixture of the previous intermediate (5.3 g, 22.4 mmol) and hexamethylenetetramine (4.7 g, 33.6 mmol) in 60 mL of chloroform was allowed to stir at room temperature overnight. On cooling of the reaction mixture in ice-water, *N*-(*trans*-4-phthalimido-2-butenyl)hexamethylenetetraminium chloride separated as a white solid (4.0 g): ^{1}H NMR (CD_3OD) δ 3.58 (d, *J* = 8 Hz, 2H), 4.4 (d, *J* = 6 Hz, 2H), 4.57 (d, *J* = 13 Hz, 6H), 4.7 (d, *J* = 13 Hz, 6H), 5.9 (m, 1H), 6.2 (m, 1H), 7.8 (m, 4H). ^{13}C NMR (CD_3OD) δ 40.0, 58.4, 72.0, 79.9, 118.7, 124.2, 133.3, 135.5, 139.8, 169.4. A solution of the latter salt (3.9 g, 10.3 mmol) in a mixture of ethanol (100 mL) and con. HCl (5 mL) was heated at reflux for 2 h. On cooling of the solution in ice-water, the precipitate was filtered off. The filtrate was concentrated to give 2.5 g of *trans*-4-phthalimido-2-butenamine·HCl (**23·HCl**): ^{1}H NMR (CD_3OD) δ 3.6 (d, *J* = 6 Hz, 2H), 4.35 (d, *J* = 4 Hz, 2H), 5.8 (dt, *J1* = 6 Hz, *J2* = 15 Hz, 1H), 6.0 (dt, *J1* = 5 Hz, *J2* = 15 Hz, 1H), 7.8–7.9 (4H). Without further purification, triethylamine (1.8 g, 18 mmol) was added slowly to a solution of **23·HCl** (2.5 g, 10 mmol) in 50 mL of ice-cold methanol, followed by dropwise addition of a solution of acetic anhydride (1.8 g, 24 mmol) in 10 mL Et_2O. The mixture was stirred at room temperature for 4 h. After the solvent was removed, the residue was extracted with EtOAc and washed with water. The organic layer was dried over Na_2SO_4 and concentrated. The residue was recrystallized from *i*-PrOH–Et_2O to give 0.2 g of the *N*-acetyl derivative of **23**. ^{1}H NMR ($CDCl_3$) δ 1.9 (s, 3H), 3.85 (t, 2H), 4.3 (d, 2H), 5.7 (m, 2H), 6.15 (br s, 1H), 7.73 (m, 2H), 7.86 (m, 2H). A solution of the latter acetamide (0.2 g, 0.8 mmol) in 10 mL of ethanol was heated at reflux with hydrazine (0.025 g, 0.8 mmol) for 3 h. The reaction mixture was cooled to room temperature, the precipitate was filtered off, and the filtrate was concentrated. The residue was dissolved in methanol and subjected to flash chromatography using EtOAc–MeOH (1:1) as eluent to give 0.1 g of **16**. **16** HCl salt: mp 182–184 °C. ^{1}H NMR (CD_3OD) δ 2.2 (s, 3H), 3.58 (d, *J* = 6 Hz, 2H), 3.95 (d, *J* = 5 Hz, 2H), 5.8 (m, 1H), 5.95 (m, 1H). ^{13}C NMR (CD_3OD) δ 21.4, 41.7, 42.5, 125.1, 133.2, 175.0. HRMS calcd for $C_6H_{10}NO$ ($[M-NH_2]^+$) *m*/*z* 112.0725, found 112.0762 (94%).

***Z*-4-Amino-2-chloro-1-(dimethylamino)-2-butene dihydrochloride (17) and *Z*-4-amino-3-chloro-1-(dimethylamino)-2-butene dihydrochloride (18).** Compound **11** (450 mg, 2.4 mmol) was heated at reflux in a mixed solution of concd HCl (15 mL) and glacial acetic acid (15 mL) for 1 week. After evaporation of volatile materials, the ^{1}H NMR spectrum of the remaining residue showed **17** and **18** as the only products in a ratio of 7:10. To the mixture of **17** and **18** dissolved in 30 mL of THF–water (2:1), was added sodium hydroxide (960 mg, 24 mmol) and di-*tert*-butyl dicarbonate (780 mg, 36 mmol), and the solution was stirred overnight at room temperature. The reaction mixture was extracted with EtOAc (30 mL×2), and the organic layer was dried over Na_2SO_4, concentrated, and subjected to column chromatography using EtOAc–MeOH (9:1) as eluent to obtain faster moving *t*-Boc derivative **24** (120 mg, 0.48 mmol) and slower moving *t*-Boc derivative **25** (160 mg, 0.65 mmol). Compound **24**: ^{1}H NMR ($CDCl_3$) δ 1.37 (s, 9H), 2.17 (s, 6H), 2.98 (s, 2H), 3.83 (t, *J* = 5.99 Hz, 2H), 4.99 (br s, NH, 1H), 5.72 (t, *J* = 5.99 Hz, 1H). ^{13}C NMR ($CDCl_3$) δ 28.4 (3C), 38.0, 44.8 (2C), 66.3, 79.4, 126.0, 133.4, 155.9. Compound **25**: ^{1}H NMR ($CDCl_3$) δ 1.42 (s, 9H), 2.22 (s, 6H), 3.10 (d, *J* = 6.48 Hz, 2H), 3.88 (d,

$J = 5.61$ Hz, 2H), 5.17 (br. s, NH, 1H), 5.80 (t, $J = 6.39$ Hz, 1H). ^{13}C NMR ($CDCl_3$) δ 28.4 (3C), 45.0 (2C), 47.2, 56.7, 79.8, 123.3, 133.6, 155.5. Compounds **24** and **25** were individually treated with 1 mL of concd HCl and evaporated to give **17·HCl** and **18·HCl**, respectively. Compound **17·HCl**: ^1H NMR (D_2O) δ 2.95 (s, 6H), 3.92 (t, $J = 6.95$ Hz, 2H), 4.18 (s, 2H), 6.41 (t, $J = 6.59$ Hz, 1H). ^{13}C NMR (D_2O) δ 37.3 (−), 42.5 (2C, +), 62.6 (−), 128.6 (+), 128.8 (−). HRMS calcd for $C_6H_{13}{}^{35}ClN_2$ (M^+) and $C_6H_{10}{}^{35}ClN$ ($M^+ - NH_3$) *m/z* (rel. intensity) 148.0767 and 131.0502, found 148.0776 (<1%) and 131.0504 (45%), respectively. Compound **18·HCl**: ^1H NMR (D_2O) δ 2.93 (s, 6H), 4.02 (s, 2H), 4.04 (t, $J = 6.95$ Hz, 2H), 6.32 (t, $J = 7.08$ Hz, 1H). ^{13}C NMR (D_2O) δ 42.9 (2C, +), 45.6 (−), 54.5 (−), 121.5 (+), 134.9 (−). HRMS calcd for $C_6H_{13}{}^{35}ClN_2$ (M^+) and $C_6H_{10}{}^{35}ClN$ ($M^+ - NH_3$) *m/z* (rel. intensity) 148.0767 and 131.0502, found 148.0758 (6%) and 131.0528 (19%), respectively.

Enzymology

Time-dependent inactivation of BPAO by candidate inhibitors was determined as in our recent studies,[24,28] by taking aliquots over time of primary incubations of BPAO with inhibitors and assaying for the conversion of benzylamine (10 mM) to benzaldehyde at 250 nm. Inactivation time courses were determined twice for most of the concentrations used, though the time points were non-identical, and thus activity loss profiles were not statistically evaluated. The figures shown are representative plots where the plateau activity was found to vary by ±5% between runs. In the determination of partition ratios, the points at high [I]/[E] were neglected in selecting the best straight line.[20] Irreversibility of BPAO inhibition was checked by gel filtration[24] or after 24 h dialysis, with the before and after activity assays varying no more than 5% in the cases of irreversible inhibitors. Phenylhydrazine titration of the active site[22] after incubation of BPAO with irreversible inactivator was performed as described[24,28] on dialyzed or gel-filtered preparations that showed less than 8% activity. The nitroblue tetrazolium (NBT) redox cycling assays[23] were performed as described[24,28] on denatured preparations of enzyme that showed less than 7% activity. No alteration of the electrophoretic properties of the enzyme or the intensity of Coomassie staining was observed for the gel run in parallel.

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