Neighboring Amide Participation in the Fenton Oxidation of a Sulfide to Sulfoxide, Vinyl Sulfide and Ketone in Spatially Restricted Methionine Analogues

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ABSTRACT

Oxidation of Met affects the stability of proteins, and was identified as a step in the beta amyloid-dependent pathogenesis of Alzheimer's disease. One-electron oxidation of Met is facilitated through stabilization of sulfide radical cations with electron-rich heteroatoms. The formation of such 2-center-3-electron bonds, formed between sulfide radical cations and amides, leads to pronounced product selectivity during biologically relevant oxidation conditions. Conformationally constrained methionine analogs embedded within a norbornane framework, i.e., 2,6-*endo*, *endo*- and 2,6-*exo*, *endo*- pyrrolidine amide thiomethyl bicyclo[2.2.1]heptanes

were synthesized. Oxidation of both methionine analogs in the Fenton oxidation yielded some sulfoxide. In addition, the oxidation of the endo, endo-derivative generated a vinyl sulfide while the exo, endo-derivative was converted into a ketone, indicating a selective influence of a sulfur-oxygen 2-center-3-electron bond on product formation. Mechanistic details of product formation were investigated through the incorporation of stable isotopes.

KEYWORDS

Two center–three electron (2c-3e) bond, sulfide radical cation, conformationally constrained methionine, Fenton oxidation, neighboring group effect

1. Introduction

Redox processes of methionine (Met) have attracted considerable attention in the biomedical sciences, as protein Met oxidation can affect enzyme activity,^{1,2} protein turnover,³ and/or protein-protein interactions.^{4,5} Methionine (Met) serves important functions in proteins including (i) the maintenance of conformational stability, e.g. through interaction of the sulfur with π -systems,⁶ (ii) the binding of transition metal-containing ligands, and (iii) the control of redox processes.⁷⁻¹⁰ Met residues in proteins have been referred to as endogenous antioxidants,¹¹ based on their facile oxidation to Met sulfoxide,^{12,6} which can be reversed through the methionine sulfoxide reductase enzymes.

In particular, the metal-catalyzed one-electron oxidation reaction of Met appears to play a role in the neurotoxicity of β -amyloid peptide (β AP),^{13,14} involved in the pathogenesis of Alzheimer's disease. However, the molecular features of β -amyloid peptide, which supports the one-electron oxidation of Met are poorly understood. This motivated our experimental work on metal-

catalyzed oxidation of specifically designed organic Met analogs, described in the present paper. β AP contains a Met residue at position 35, Met[35], which appears to be involved in the generation of reactive oxygen species through the reduction of Cu^{2+} .^{15,14,16-20} Thermodynamically, the one-electron oxidation of Met by β AP-bound Cu^{2+} is unfavorable.^{11,21,22} However, the one-electron oxidation of Met by Cu^{2+} (eq 1) may be driven by exergonic follow-up reactions, such as the deprotonation of an intermediary sulfide radical cation,^{23,24} representatively shown for the Met ϵ - CH_3 group (eq 2).

In addition, any reduction of the one-electron reduction potential of Met may promote the one-electron oxidation by Cu^{2+} . Theoretical calculations on β AP, where Met[35] is incorporated into a C-terminal α -helix (based on the NMR structure of β AP1-40 in a micelle-containing aqueous solution²⁵), show that the one-electron oxidation of Met[35] can result in bond formation between the Met sulfur and the oxygen of the peptide bond C-terminal of Ile[31].²⁶ Such sulfur-oxygen bond formation may result in the stabilization of a sulfide radical cation²⁷ and, consequently, reduce the oxidation potential of Met[35]. Time-resolved pulse radiolysis experiments have, indeed, provided evidence for the association of a sulfide radical cation with the peptide bond in model compounds such as N-acetyl-Met amide,²⁸ but also in a heptapeptide, N-Ac-Gly₃MetGly₃.²⁹ Importantly, when the C-terminal α -helix of β AP1-40 was disrupted through substitution of Ile[31] by Pro[31], the mutant β AP1-40(Ile[31]Pro) had a significantly lower efficiency at reducing Cu^{2+} as compared to wild-type β AP(1-40).³⁰ The mutant also showed a ca. 10-fold reduced efficiency to form a sulfur oxygen bond between Met[35] and

Pro[31] as compared to Met[35] and Ile[31] in wild-type β AP(1-40),²⁶ consistent with a contribution of sulfur-oxygen bond formation to the oxidation of β AP(1-40) by Cu^{2+} .

The sulfur-oxygen bond formed between a sulfide radical cation and a peptide bond oxygen is best characterized as a 2-center-3-electron (2c-3e) bond, indicated through the symbol $\text{S} \cdot \cdot \text{O}$.³¹

For a better characterization of such sulfur-oxygen three-electron bonds we applied pulse radiolysis to synthetic substituted norbornane derivatives,³² including compounds **1** and **2**,³³ where the

pyrrolidine amide substituent was either in the endo (**1**) or exo (**2**) position. These compounds were subjected to oxidation by hydroxyl radicals ($\text{HO}^\bullet$), and time-resolved UV-spectroscopy used to monitor the formation of three-electron sulfur-oxygen bonded species. Clear spectroscopic evidence for a sulfur-oxygen three-electron bond was obtained for **1** while such species could not be detected for species **2**. These results confirmed earlier pulse radiolysis experiments with simple amides.³² Importantly, certain S-substituted endo norbornanes showed significantly lower oxidation potentials as compared to exo-substituted norbornanes, confirming a role of sulfur-oxygen bond formation in the lowering of these potentials.^{32,33}

It can be expected that the formation of three-electron bonded sulfur-oxygen species is of relevance to product formation upon one-electron oxidation of Met derivatives, or, more generally, Met residues in proteins. Such formation of a sulfur-oxygen bond would rationalize

the high propensity for β -amyloid peptide Met oxidation, associated with the formation of reactive oxygen species.^{15,13,16-18} Therefore, we subjected the model compounds **1** and **2** to oxidation by well-defined Fenton chemistry, and, for comparison, to electrochemical oxidation, and analyzed the products by HPLC-MS/MS using high-resolution mass spectrometry.

We shall demonstrate that the Fenton oxidation of 2,6-*endo,endo*- and 2,6-*exo,endo*- pyrrolidine amide thiomethyl derivatives of norbornane lead to the formation of sulfoxide, vinyl sulfide and/or ketone derivatives where the formation of the vinyl sulfide (and the ketone) are dependent on the intermediary formation of an ($>\text{S}:\cdot\text{O}<$)⁺ bond.

1. Results

The products formed on Fenton oxidation of compounds **1** and **2** were separated by LC. The structures of the products formed were determined by a combination of methods: MS, MS/MS analysis of gas-phase fragmentation pathways, chemical derivatization and comparison with chemically synthesized compounds. MS fragmentation pathways were based on literature precedent and theoretical calculations. A signature pathway has been identified as the retro-Diels-Alder reaction (RDA) if a double bond is present in the norbornyl ring or generated in a fragmentation pathway. This latter process is well illustrated in MS/MS analysis of compounds **1** and **2**. Their MS/MS spectra, analysis and proposed gas-phase fragmentation are presented in the Supporting Information (Fig. S1-S4).

2.1. Fenton oxidation of **1**

2.1.1. LC-MS analysis

Shown in Fig. 1 is the LC-MS analysis of the products obtained by Fenton oxidation of **1**. In addition to unreacted **1** two products **3** and **4** were found. The structures of these products were determined by a combination of methods as presented below.

Fig. 1: LC-MS analysis of the oxidation products obtained after metal-catalyzed oxidation of **1** and **2**. (A) compound **1**; (B) oxidation of **1** by the Fenton reaction after 60 min of incubation at 37°C; (C): compound **2**; (D) oxidation of **2** by the Fenton reaction after 60 min of incubation at 37°C.

2.1.2. CID of **3**

The CID of **3** with assignments of the proposed structures of the major fragments is shown in Fig. 2. Since the parent ion of **3** has m/z 256.2 which is 16 mass units greater than **1** (m/z 240.2) it is reasonable to assume that **3** is the sulfoxide corresponding to **1**. However, this requires proof. The MS/MS spectrum of **3** displays nine fragment ions with m/z 91.12, 93.12, 119.07, 121.12, 126.15, 139.13, 157.13, 185.13, and 192.21 (Fig. 2A). The fragment ions with m/z 93.12, 126.15, and 192.21 are common to the CID spectra of both product **3** and compound **1**, and are, therefore, mechanistically related to the pathways described in Fig. S3. The formation of these ions during CID of **3** demonstrates that i) no oxygen is attached to any carbon of the norbornane structure (ion with m/z 93.12), ii) the oxidation of **3** does not occur on the pyrrolidine function, since the ion with m/z 126.15 is observed, and iii) the oxidation has to occur on the methyl-thio group, since we can observe the ion with m/z 192.21, resulting from the neutral loss of 64 Da (corresponding to the loss of CH_3SOH) from the parent ion (m/z 256.2). During their introduction into the mass spectrometer, the samples are heated to allow their volatilization. The samples are heated at temperatures between 100°C and 200°C, corresponding to the source and desolvation temperatures, respectively. Therefore, the formation of the ion with m/z 192.21 might also be the result of a thermal loss of CH_3SOH from **3**. Thermolysis of sulfoxides to yield alkenes and sulfenic acids is well-known.^{34,35} The remaining fragment ions confirm the formation of the sulfoxide. First, the ion with m/z 185.13 corresponds to a mass increase of 16 Da compared to the ion with m/z 169.13, which was obtained during the CID of **1** (Figure S1). Second, the ion with m/z 185.13 can fragment according to two different pathways as outlined in Fig. 3 (pathways A and B). The oxonium ion (m/z 185.13, Fig. 3A) allows for the loss of CO (MW = 28 Da) leading to the formation of an ion with m/z 157.13 (Fig. 3A). The difference of

mass between the ions with m/z 157.13 and m/z 139.13 is 18 Da, suggesting that a molecule of H_2O is eliminated from the fragment ion with m/z 157.13. After the loss of CO, the ion with m/z 157.13 can rearrange into the intermediate ion **5**. The endo proton shown on C3 can be transferred to the sulfoxide moiety. This mechanism permits the formation of a carbon-carbon double bond between C2 and C3 and the protonation of the sulfoxide group affording ion **6**. Finally, the release of H_2O from the intermediate ion **6** permits the formation of the ion with m/z 139.13. Alternatively, intermediate ion **6** undergoes a RDA fragmentation, which generates the ion with m/z 91.12.

Fig. 2: CID of **3** obtained after Fenton oxidation of **1** in H_2^{16}O (A, m/z of the parent ion 256.2), and in H_2^{18}O (B, m/z of the parent ion 258.2).

The ion with m/z 185.13 (Fig. 3A) can also cyclize to form the intermediate ion **7**. The cyclic structure in **7** allows for transfer of the endo proton located at C5 onto the sulfur atom. Such reaction permits the formation of the intermediate ion **8**, which contains a carbon-carbon double bond between the carbon atoms C5 and C6 and permits the release of methanesulfenic acid (CH_3SOH) and the formation of an ion with m/z 121.12. Sulfenic acid derivatives have been documented as possible fragment ions during CID of 2-dimethylamino-ethanesulfoxide.³⁶ In the ion with m/z 121.12 (Fig. 3B), the presence of the acylium ion allows for the release of CO and the formation of the ion with m/z 93.12 (Fig. 3B). DFT calculations to support these fragmentation mechanisms will be presented below.

Fig. 3: Proposed reaction pathways of the gas-phase fragmentation of **3** during MS/MS analysis.

The difference of mass between the ions with m/z 119.07 of **3** (Fig. 3C) and m/z 103.07 of **1** (Fig. S3) is 16 Da. This difference suggests that the ion with m/z 103.07 is the result of the fragmentation of the sulfoxide **3**. Thus, we hypothesize that the structure of the ion with m/z 119.07 (Fig. 3C) should be similar to that of the ion with m/z 103.07 (Fig. S3D) except for an additional oxygen atom. The intermediate ion **8** (Fig. 3B) can generate the ion with m/z 119.07 (Fig. 3C) through a RDA fragmentation. The RDA fragmentation of **8** is consistent with the observation that the ions with m/z 103.07 and m/z 119.07 differ only by the presence of one oxygen atom. The fragmentation pathways were confirmed by Fenton oxidation in H_2^{18}O , which leads to partial ^{18}O -incorporation into the sulfoxide product **3** (see also below). Fig. 2B shows that specifically the fragments containing an intact sulfoxide function showed the expected increase of two atomic mass units. Oxidation of **1** can give two diastereomeric sulfoxides; however, only one peak is observed in the chromatogram. Thus, either the oxidation is diastereoselective (like the sodium metaperiodate oxidation; see below) or the two diastereoisomers of **3** co-elute.

2.1.3. Theoretical calculations of fragment ion energies

All the structures presented in Fig. 3 were optimized at the B3LYP/6-31G(d) level. The energetic diagram presented in Fig. 4 shows the relative ΔG in kcal/mol of the reactions presented in Fig. 3. All ΔG values were calculated relative to the most stable structure **5** (which was arbitrarily assigned $\Delta G = 0$). Using thermometer ions (para-substituted benzylpyrrolidinium salts), De Pauw et al. established a correlation between the volt-rampage applied to parent ions during their CID gas-phase fragmentation and the internal energy of the fragment ions generated in triple-

quadrupole and FT-ICR mass spectrometer instruments.³⁷⁻³⁹ For a voltage of 35 V, as used during our MS/MS experiments, we cannot expect more than 50 kcal/mol of internal energy transfer to the fragment ions. Therefore, the fragmentation reactions considered in Fig. 3 excluded any reaction that would require more than 50 kcal/mol to generate a new intermediate fragment ion. The calculations clearly indicate that i) the intermediate ions **5** and **7** are the most stable ions and ii) the ΔG of formations of all the ions resulting from the fragmentation of the intermediates **5** and **7** require less than 50 kcal/mol. Therefore, the structures of the ions outlined

in Fig. 3 represent the most probable ion structures that could be generated in gas-phase during CID of **3**.

Fig. 4. DFT calculations of the intermediates presented in Scheme 4. The geometry optimization and frequency calculations were carried out at the B3LYP/6-31G(d) level by the hybrid HF-DFT procedure implemented in the Gaussian software package. The geometries of the molecules were

optimized in vacuum. Energies are given in kcal/mol. The numbers in brackets refer to the reaction pathways presented in Fig. 3.

2.1.4. Chemical synthesis of **3**

Oxidation of thioether **1** to the corresponding sulfoxide was achieved using sodium metaperiodate in aqueous methanol.⁴⁰ Selective oxidation occurred and recrystallization of **3** gave a pure diastereomeric sulfoxide whose structure including stereochemistry was unequivocally established by an X-ray crystallographic structure study. An ORTEP drawing of this sulfoxide is shown in the Supplementary Material (Fig. S5). This chemically prepared compound and that formed in the Fenton reaction were shown to be the same by chromatography and mass spectrometry analysis.

2.1.5. CID of **4**

After Fenton oxidation of **1**, the samples were fractionated by flash chromatography on a C18 column (5 cm x 4.6 mm, 3.5 μ m, Grace, Deerfield, IL) using H₂O (A, 100%) and ACN (B, 100%) as aqueous and organic eluents, respectively. The samples were eluted at 1.0 mL/min through the column using a step gradient of 100% of A for 5 min followed by 100% of B for 10 min. The collected fractions were lyophilized. After lyophilization, the fractions were reconstituted in 1 mL of EtOD (deuterated ethanol):D₂O (deuterium oxide) (1:1, v:v), which contained 10 mM hydrazine. The samples were left overnight at room temperature prior to lyophilization. The samples were then reconstituted in milliQ H₂O for LC-MS analysis.

Product **4** is eluted at t_{el} = 5.8 min and its molecular ion with m/z 238.20 indicates a loss of 2 Da compared to the original compound **1** (Fig. 1B). The MS/MS spectrum of **4** (Fig. 5A) is

consistent with the structure shown below (i.e., the norbornene derivative of **1** with a double bond between C5 and C6 of the original norbornane).

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During CID of **4**, the parent ion can enter immediately into a RDA- fragmentation, shown below, justifying the unique formation of any additional ion with m/z 126.15, and the absence of the fragment ion with m/z 192.21 (Fig. 5A).

After subjecting **1** to Fenton oxidation in the presence of D_2O , the mass spectrometry analysis of product **4** revealed the incorporation of one equivalent of deuterium (Fig. 5B vs 5C). The MS/MS analysis of the deuterated product **4** did not reveal any incorporation of deuterium into the fragment ion with m/z 126.15 (Fig. 5A), indicating that neither the carbons C2 and C3 nor the pyrrolidine group contain the deuterium. Instead, the deuterium atom must be incorporated at

either C1, C4, C5, C7, or the methyl group. A rationale for the deuterium incorporation during the Fenton oxidation of **1** will be provided below.

Fig. 5. (A) CID fragmentation of **4**, obtained after selecting the parent ions with m/z 238.14 (see panel C) or with m/z 239.14 (see panel B). (B) Isotopic distribution of product **4** obtained after Fenton oxidation of **1** in the presence of D_2O . (C) Isotopic distribution of product **4** obtained after Fenton oxidation of **1** in the presence of H_2O

2.1.6. Chemical derivatization of **4**

The structural assignment of product **4** was further supported through *syn*-deuteration of **4** by dideuterodiimide (DN=ND) generated by copper-catalyzed oxidation of tetradeuterohydrazine (D₂N-ND₂) in D₂O. This reaction is expected to generate the doubly deuterated compound **1**, with a molecular ion of m/z 242.2. LC-MS analysis of the deuterated product of **4** reveals the formation of a product with m/z 242.2 (Fig. 6B and C). However, the product with m/z 242.2 does not show the same elution time as the original compound **1** during HPLC analysis (t_{el} = 14.5 min, Fig. 6A), but, instead, an elution time of t_{el} = 17.5 min (Fig. 6C). Such elution time corresponds to the elution time of compound **2** rather than **1** (Fig. 6A vs B). These observations suggest that during the deuteration of **4** a product is generated, which displays a molecular surface area more comparable to that of compound **2** rather than compound **1**. The rearrangement of the endo methylthio-substituent to an exo methylthio-substituent, such as displayed in the tentative structure **9**, is consistent

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with such change in surface area. The relationships between molecular surface areas of hydrocarbons and their reversed-phase retention behavior were documented by Möckel et al,⁴¹ indicating a significant influence of molecular surface area on chromatographic retention. If indeed **9** is the product of reduction, it may be conjectured that the endo-amide group directs the diimide for endo-attack rather than the expected sterically preferred exo attack in analogy with the reported^{42,43} directing effect of an acetate moiety in diimide reduction. Noteworthy, the formation of **9** is not very efficient, displaying a yield $\leq 10\%$ relative to **4**. This low yield explains why an ion search of the entire scan is required to detect the formation of **9** (Fig. 6C). The low yield of transformation of **4** into **9** may indicate that also other, non-reducible products with m/z isobaric to that of **4** may be formed.

Fig. 6: A) LC-MS analysis after Fenton oxidation of **1** (m/z 240.2). B) LC-MS analysis after Fenton oxidation of **2** (m/z 240.2). C) Ion search for product **9** (m/z 242.2) which was obtained after Fenton oxidation of **1** and hydrogenation of product **4** (m/z 238.2) in the presence of D_2O .

2.2. Fenton oxidation of **2**

2.2.1. LC-MS analysis

Shown in Fig. 1D is the LC-MS analysis of the products obtained by Fenton oxidation of **2**. In addition to unreacted **2**, two products **10** and **11** were formed. The m/z for **10** is 256.2 which is 16 mass units over that of **2** suggesting the formation of the corresponding sulfoxide **10**. In contrast to the sulfoxide **3** found by Fenton oxidation of **1**, product **10** is eluted as a double peak with $t_{el} = 8.2$ min and 8.7 min, and corresponds to the sulfoxide of **2** (Fig. 1D). In contrast to product **3**, we are able to chromatographically separate two diastereoisomers of **10**. Indeed, a selected ion search for m/z of 256.20 from the LC-MS chromatograms obtained after Fenton oxidation of **1** and **2** allows distinguishing one peak and two peaks for the sulfoxide products **3** and **10** resulting from the oxidation of **1** and **2**, respectively (Figures 7A vs 7B).

Fig. 7: Selected ion chromatograms for m/z 256.20 extracted from the LC-MS chromatograms of the oxidation products obtained after metal-catalyzed oxidation of **1** (A) and **2** (B).

2.2.2. CID of **10**

The MS/MS analyses of the isomers of **10** show the same profiles with six major fragment ions with m/z 91.10, 93.11, 126.15, 157.13, 164.20, and 192.21 (Fig. 8). The only fragment ion, which contains the sulfoxide group is the ion with m/z 157.13. Indeed, the m/z of this ion corresponds to an increase of 16 Da compared to the ion with m/z 141.13, which is also observed during the CID of **1**.

Fig. 8: CID of **10**.

Importantly, the ion with m/z 157.13 is observed as a minor fragment ion during the CID of **3** (Fig. 2), but as the second most intense fragment ion during the CID of **10**. We know from the

MS/MS of **3** that the ion with m/z 157.13 is the result of the loss of CO from the ion with m/z 185.13 (Fig. 1 and 3A). In our proposed fragmentation pathways of **3**, the ion with m/z 185.13 (Fig. 3A) was stabilized by the presence of the juxtaposed sulfoxide group. Indeed, the endo orientations of both the sulfoxide moiety and acylium ion allowed for stabilization of the acylium ion by formation of cyclic ion **7** (Fig. 3), which delayed the loss of CO. In the case of **10**, we can only hypothesize that an intermediary ion with m/z 185.13 is formed, but could not be observed, because of the immediate loss of CO, leading to the ion with m/z 157.13. This can be rationalized by the exo position of the pyrrolidine amide moiety of **10**. Because the sulfoxide remains in the exo position of C6 of the norbornane, stabilization of the acylium ion by the SO group is geometrically precluded. Therefore, the CO group is instantly lost to form the ion with m/z 157.13 as shown below:

The ions with m/z 126.15, 164.20, 192.21 and 91.11, 93.11 can be explained by fragmentation mechanisms analogous to those described in Figs.3 and S4. As observed already during the CID of **2**, the ion with m/z 126.15 is the major fragment ion. The high abundance of this ion is likely due to a favorable configuration (the pyrrolidine amide moiety at the equatorial position of the norbornane) for a retro-Diels-Alder fragmentation, as described in Fig. S4A.

2.2.3. Chemical synthesis of 10

Oxidation of **2** was achieved using sodium metaperiodate in aqueous methanol³⁵ to give the sulfoxide as a mixture of diastereomers. This chemically prepared mixture of sulfoxide diastereomers and that formed in the Fenton reaction were shown to be the same by chromatography and mass spectrometry analysis.

2.2.4. CID of 11

LC-MS analysis of the Fenton oxidation of **2** produced **11** eluted at $t_{el} = 8.5$ min with m/z 208.2 which is 32 mass units below that of **2** (Fig. 1D). This suggests loss of conversion of CHSMe into C=O. This product is not formed during the Fenton oxidation of compound **1**. The CID of **11** is shown in Fig. 9.

Fig. 9. CID fragmentation of **11**.

The proposed fragmentation mechanisms of **11** during MS/MS analysis (Fig. 12) are displayed in Fig. 10. Product **11** is the result of the oxidation of **2**, therefore, the RDA fragmentation observed during the CID of **2** should also be possible during the fragmentation of **11**. Enolization of **11** generates the precursor for the RDA fragmentation (Fig. 10A) generating the ion with m/z 126.15. The formation of the ions with m/z 109.08 and 91.06 can be explained through the fragmentation pathway B described in Fig. 10. The ions with m/z 81.08 and m/z 67.05 are rationalized by the fragmentation pathway C in Fig. 10.

Since **11** appeared to be a ketone a standard ketone derivative was prepared and analyzed as follows.

Fig. 10.: Proposed reaction pathways for the gas-phase fragmentation of **11** during MS/MS analysis.

2.2.5. Chemical derivatization of 11

After Fenton oxidation of **2**, the samples were fractionated by flash chromatography as described above. After lyophilization, some aliquots of the samples were reconstituted in milliQ H₂O at pH 2 in the presence of 2,4-dinitrophenylhydrazine (DNPH, 2 mM). The pH was adjusted by 1M HCl solution. After one hour of reaction at room temperature, the reaction products were analyzed by LC-MS. Two new peaks were observed during LC-MS analysis (Fig. 11A vs B).

Fig. 11. LC-MS analyses of the oxidation products obtained after metal-catalyzed oxidation of **2** (A), and after exposure of the products of oxidation to 2,4 dinitrophenyl hydrazine (B).

These peaks eluted at $t_{el} = 10.5$ min and at $t_{el} = 22.0$ min, and correspond to unreacted DNPH and to product **12**, respectively (Fig. 11B). Product **12** represents the hydrazone of **11**, with a molecular ion with m/z 388.15. The MS/MS analysis of **12** allows for the identification of the hydrazone at the original C6 of **2** (Fig. 12A). During the CID of **12** in particular the fragment ions with m/z 205.23 and m/z 166.20 are characteristic of the fragmentation of the hydrazone (Fig. 12A). The formation of the 6-imino-3-nitro-N-oxocyclohexa-2,4-dieniminium ion (m/z 166.20) confirms the formation of the hydrazone. Moreover, the presence of the ions with m/z 126.15 and m/z 98.12, which are also observed during RDA fragmentation of **2** (Fig. S4A), confirm that the DNPH group is not attached on the side of the pyrrolidine moiety. Thus, the fragmentation of **12** is consistent with the ketone function being located on the carbon atoms C5 or C6 of the norbornane.

Fig. 12. (A) CID fragmentation of **12**. (B) Ion search for the ion with m/z 208.2 from the LC-MS analysis of the synthesized **11**. (C) Ion search for the ion with m/z 208.2 from the LC-MS analysis obtained after Fenton oxidation of **2**.

2.2.5. Chemical synthesis of 11

To complete the characterization of products **11** and **12**, we have synthesized **11**, by the chemical route shown in Fig. 13, fully characterized it, and compared its elution and its MS/MS profile with that of product **11** obtained after the Fenton oxidation of **2**.

Fig. 13.: Synthesis of bicyclo[2.2.1]heptan-2-one-6-endo-pyrrolidineamide (**17**) and bicyclo[2.2.1]heptan-2-one-6-exo-pyrrolidineamide (**11**).

Epimerization of the known endo-ester ketal **14**⁴⁴ with sodium methoxide afforded predominantly the exo-acid ketal **15** (Fig. 13B) which was hydrolyzed quantitatively with aqueous hydrochloric acid to keto exo acid **16** (Fig. 13C). The structure of the keto exo acid **16** was established by IR, ¹H and ¹³C NMR spectroscopic analyses. This intermediate was converted to keto exo-amide **11** by sequential reaction with oxalyl chloride followed by pyrrolidine (Fig. 13D). The structure of **11** was established by spectroscopic analysis and high resolution mass spectrometry. The results clearly show that the synthetic **11** elutes at the same time as Fenton product **11** (Fig. 12B vs 12C). Moreover, after derivatization of the synthetic **11** with DNPH, the hydrazone shows the same mass spectrometric properties as **12**: i) a molecular ion with m/z 388.2, ii) an elution time at $t_{el} = 22.0$ min, and iii) an MS/MS fragmentation profile identical to the one presented in Fig. 12A.

To ensure that the exo and endo keto-amides could be distinguished and that no endo keto amide is formed on the Fenton oxidation of **1**, endo keto amide **17** was also prepared as shown in Fig. 13. Treatment of a solution of the known ester **13**⁴⁵ in toluene with pyrrolidine under reflux afforded the keto endo pyrrolidine amide **17** in 34% yield (Fig. 13A). The crystalline compound was fully characterized spectroscopically and by high resolution mass spectrometry.

2.3 Time dependence of the Fenton oxidation of compounds **1** and **2**

Compounds **1** and **2** were subjected to Fenton oxidation over a period of 60 min according to the experimental protocol described in the Experimental section. The relative amounts of the oxidation products⁴⁶ (**3**, **4**, **10**, and **11**) are reported after 0 min, 5 min, 30 min, and 60 min (Fig. 14A and B). Over 30 min of oxidation, the oxidation rates of compounds **1** and **2** are rather

similar (Fig. 14A and B, ●). Of importance, the oxidation can be divided into a fast (completed by ca. 5 min) and a slow process (at times > 5 min). Such behavior has been observed before during the Fenton oxidation of the dipeptide Met-Met,⁴⁷ and rationalized by a Fe^{II}-dependent fast oxidation and a Fe^{III}-dependent slow oxidation process. Indeed, after 5 min, and 30 min of oxidation, 24%, and 33%, of compound **1** were transformed into both products **3** and **4**, while 19%, and 26%, of compound **2** were transformed into products **10** and **11**, respectively. Interestingly, after 60 min. of oxidation, **1** continues to degrade into the sulfoxide product **3** (at 60 min, 41% of **1** is essentially converted into **3**, Fig. 14, ■) while **2** does no longer degrade (at 60 min, 28% of **2** is converted into both products **10** and **11**, Fig. 14, ■, ▲). The formation of the sulfoxide product **3** is linear with a slope of 0.6 min⁻¹ (Fig. 13, A, ■). Product **4** reaches a maximum after 5 min of oxidation (Figure 14, ▲), before it disappears over the next 55 minutes of oxidation. Product **3** is therefore mostly generated in the slow process while **4**, **10**, and **11** are generated in the fast process.

Fig. 14. Time dependence on the Fenton oxidation of compounds **1** and **2**. (A) Fenton oxidation of **1** (●), formation of products **3** (■) and **4** (▲). (B) Fenton oxidation of **2** (●), formation of products **10** (■) and **11** (▲).

2.3. Fenton oxidation of compounds **1** and **2** in H_2^{18}O

Compounds **1** and **2** were oxidized in $\text{H}_2^{18}\text{O}:\text{H}_2^{16}\text{O}$ (75%:25%, v:v) according to the protocol described in the Experimental section. After LC-MS analysis and separation of the oxidation products, the isotopic distributions of products **3**, **10**, and **11** were compared to those obtained after formation of products **3**, **10**, and **11** in 100% H_2^{16}O (Fig. 15). The isotopic distributions

allow for calculation of the percentage of molecules, which incorporated one atom of ^{18}O into the respective products. We calculate that products **3**, **10**, and **11** in $\text{H}_2^{18}\text{O}:\text{H}_2^{16}\text{O}$ (75%:25%, v:v) incorporated one ^{18}O atom in 29.7%, 17.7%, and 25.5%, of the molecules, respectively. Hence, based on the applied ratio of $\text{H}_2^{18}\text{O}:\text{H}_2^{16}\text{O}$ (75%:25%, v:v) only 40%, 24%, and 34% of the available ^{18}O atoms are incorporated in products **3**, **10**, and **11**, respectively, indicating the operation of several parallel reactions for product formation. Control experiments show no ^{18}O exchange when **3** was dissolved in H_2^{18}O .

Fig. 15. Fenton oxidation of compounds **1** and **2** in the presence of $\text{H}_2^{18}\text{O}:\text{H}_2^{16}\text{O}$ (75:25, v:v). Comparison of the isotopic distributions of products **3** (A, B), **11** (C, D) and **10** (E, F) generated in H_2^{16}O (100%, v) and in $\text{H}_2^{18}\text{O}:\text{H}_2^{16}\text{O}$ (75%:25%, v:v). H_2^{18}O stands for H_2^{18}O -depleted water.

3.1. Electrolysis of **1** and **2**

Compounds **1** and **2** were oxidized at potentials of 1.3 V and 1.4 V, respectively, at a glassy carbon rotating disk electrode in acetonitrile (0.1 M NaClO₄) with a Ag⁺/Ag reference electrode. The oxidation products were analyzed by LC-MS (Fig. 16). Under the same conditions of electrolysis, **1** was oxidized into **3** with a yield of 94% (Fig. 16, A and C), whereas **2** was oxidized into **10** and **11** with a total yield (**10**+**11**) of 56% (Fig. 16B and D). Product **4** was not observed during the electrolysis of **1**, suggesting that either **4** could not be formed or **4** was further oxidized at the electrode.

Fig. 16. LC-MS analysis of the oxidation products obtained after electrolysis of **1** and **2**. (A) **1** before electrolysis; (B) **2** before electrolysis; (C) electrolysis of **1** at 1.1 V; (D) electrolysis of **2** at 1.3 V.

4. Discussion

An intriguing result from the product study is that Fenton oxidation of **1** gives thioenolether **4** but that such oxidation of **2** gives ketone **10**. How can we account for this difference?

4.1 Product formation from **2**

Consider the oxidation of **2**. In the presence of $\text{Fe}^{\text{II}}/\text{EDTA}$ and hydrogen peroxide (H_2O_2), hydroxyl radical ($\text{HO}^\bullet$)⁴⁷⁻⁴⁹ is generated as shown in eq. 3-6 and/or their metal-bond equivalent,⁴⁹ both of which are strong one-electron oxidants.

The reaction of hydroxyl radical⁵⁰ and their metal-bond equivalent⁴⁷ with organic sulfides and Met-containing peptides has been demonstrated to yield intermediates sulfide radical cations. Therefore, the Fenton oxidation of **1** and **2** with $[\text{Fe}^{\text{II}}(\text{EDTA})]^{2-}$ and H_2O_2 is expected to proceed via a well-defined sulfide radical cation. It is known that $\text{HO}^\bullet$ radicals react with the sulfur atom of a thioether to ultimately yield an intermediary sulfur radical cation (Fig. 17 and 18)^{51,52}. Such species are known to undergo two reaction pathways: (1) dimerization with a second non-oxidized thioether (Fig. 17B) and (2) deprotonation (Fig.17A). Dimerization generates a dimeric sulfur-sulfur bonded 2c-3e bond ($>\text{S} \cdot \cdot \text{S} <$)⁺ and deprotonation forms carbon-centered radicals. Such deprotonation reactions have been documented by pulse radiolysis for methionine²⁹ and norbornane^{32,33} derivatives. Under the Fenton oxidation conditions both species can be further oxidized by Fe^{III} . The initial concentration of **2** during Fenton oxidation was 0.7 mM. By

comparison to our pulse radiolysis data, such concentration of **2** is expected to generate some dimeric sulfur-sulfur bonded 2c-3e bond ($>\text{S}:\cdot\text{S}<$)⁺, on one-electron oxidation.^{32,33} This sulfur-sulfur 2c-3e bond may react with Fe^{III} (Fig. 17B), generating a dication, which then reacts with water to generate **2** and **11**. A carbon-centered radical at C6 can also be formed through a deprotonation at C6 (Fig. 17A). In the presence of H_2O and Fe^{III} , the carbon-centered radical can reduce Fe^{III} into Fe^{II} and lead to the formation of a thionium salt which is expected to be hydrolyzed by water to the corresponding ketone accounting for product **11**. The formation of the corresponding ketone does not occur on Fenton oxidation of **1**.

The Fenton oxidation of compounds **1** and **2** leads to the formation of the respective sulfoxide products **3** and **10**, but also to a set of completely different products, **4** and **11**, depending on the endo- or exo- position of the pyrrolidine-amide moiety. All reactants and oxidation products are characterized by mass spectrometry, where the CID of the isomers **1** and **2** leads to the formation of different series of ions (Fig. S1 and S2). These series of ions are rationalized through mechanisms that involve RDA fragmentations and the proximity of the amide and thioether moieties in compound **1**. In fact, the neighboring group participation of the amide in the oxidation pathway(s) of **1** is especially important for the generation of product **4**.

Fig. 17.: Proposed reaction pathways for (i) the formation of a dimeric sulfur-sulfur bonded 2c-3e bond during the oxidation of **2** and (ii) the formation of product **10**.

4.2 Product formation from **1**

Neighboring amide group participation, which is precluded in **2**, occurs concurrently or following formation of intermediary sulfur radical cation **18** (Fig. 18) generated by reaction of HO[•] radicals with the sulfur atom of the thioether moiety of **1**. Thus **19** with a 2c-3e bond ($>\text{S}:\cdot\text{O}<^+$)^{32,33,53} is produced. Since a first electron has been removed from the sulfur of the thioether moiety, the stabilization of the sulfur radical cation through the formation of a 2c-3e ($>\text{S}:\cdot\text{O}<^+$) bond in **1** (Fig. 18) lowers its oxidation potential and facilitates the removal of a second electron from the 2c-3e bond.⁵⁴ The 2c-3e bond can reduce Fe^{III} (Fig. 18), generating a dication (**20**), which transforms into sulfoxide **3** by reaction with H₂O. This reaction is analogous to our previous work with analogous acyloxysulfonium salts, which can be hydrolyzed by water attack on S (or C) providing the corresponding sulfoxide. Evidence for this mechanism is provided by the incorporation of ¹⁸O into **3** when the Fenton oxidation is carried out in H₂¹⁶O:H₂¹⁸O (25:75, v/v). However, our observation that the ¹⁸O level in **3** does not match the ¹⁸O level in water indicates at least one additional mechanism of sulfoxide formation. This is likely the Fe^{II}-catalyzed reaction of H₂O₂ with **1**, i.e. the reaction of **1** with the intermediate formed during reaction shown in eq .7⁴⁷

It is proposed that dication **20** undergoes a Pummerer-like rearrangement⁵⁵⁻⁵⁹ to give **22** via **21** (Fig. 18). Alternatively, deprotonation at C6 in **18** would afford the thionium salt. Reaction of

this thionium salt with the neighboring amide would also provide **21** (indeed this may be the mechanism for the suggested Pummerer reaction). However, since the thionium salt is not hydrolyzed to this corresponding ketone, in contrast to the hydrolysis of thionium salt, intramolecular trapping by the neighboring amide must be faster than nucleophilic water attack. Syn, endo-elimination reaction of **22** would afford the observed thioenolether **4**. Thus the reason why **1** produces thioenolether **4** whereas **2** affords ketone **11** is neighboring group participation by the amide moiety in **1** which is precluded in **2**. It should be noted that, if the rate determining step in thionium ion hydrolysis is water addition to generate a tetrahedral intermediate this addition is disfavored in **1** but not **2** for steric reasons. Since deuterium is incorporated into **4** in the presence of D_2O as noted above path B in Fig. 18 is proposed. Here D^+ attacks the thioenolether from the less hindered exo direction to afford deuterated **23** as shown. Syn, endo-elimination of **24** as with **21** affords **25** (monodeuterated **4**) as illustrated.⁶⁰

Fig. 18.: Proposed reaction pathways for the formation of products **3** and **4**.and for D⁺ incorporation⁶⁰ into **4**.

5. Conclusions

The gas-phase fragmentations of 2,6-*endo,endo*- and 2,6-*exo,endo*- pyrrolidine amide thiomethyl derivatives of norbornane (compounds **1** and **2**, respectively) and their oxidation products show interesting divergence. The proximity of the pyrrolidine amido and thioether moieties in the **1** permits the parent ion in the gas phase to enter in a specific fragmentation pathway controlled by this proximity. The comparison of the nature of the oxidation products generated during Fenton oxidation of compounds **1** and **2** in aqueous solution supports the involvement of a 2c-3e ($>\text{S}:\text{O}<$)⁺ bond in the unexpected divergent formation of products **4** and **11** which is ascribed to neighboring group amido participation preventing hydrolysis of the thionium moiety formed on oxidation of **1** but not **2**. The divergence in the formation of oxidation products arising from the conformationally constrained methionine analogs **1**, and **2**, provides new chemical pathways to explain the selectivity of oxidation reactions. For example, we have recently shown that the intermediary formation of S: $\cdot$ O bonded intermediates may lead to selective redox-mediated peptide bond hydrolysis in parathyroid hormone [(PTH)1-34].⁶¹

6. Experimental section

6.1. General information

Ammonium acetate (AAc), hydrogen peroxide (H_2O_2), hydrogen chloride (HCl), hydrazine, iron (II) sulfate heptahydrate ($\text{FeSO}_4 \times 7 \text{ H}_2\text{O}$), ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), acetonitrile (ACN), and 2,6-di-ter-butylpyridine (DTBP) were supplied by Sigma-Aldrich (St Louis, MO) at the highest available purity grade.

All reactions presented in Fig. 13 were performed in oven dried glassware under an inert atmosphere of argon unless otherwise stated. Anhydrous tetrahydrofuran (THF) was distilled from sodium metal and benzophenone and anhydrous benzene was distilled from calcium hydride and stored over activated molecular sieves. Anhydrous methanol was prepared by refluxing over magnesium methoxide for three hours and then distilled out and stored over molecular sieves. All other reagents were used as purchased without further purification. Reactions were monitored by thin layer chromatography on TLC plastic sheets silica gel 60 F254. Crude products were purified using flash grade silica gel from Dynamic Adsorbents, Inc. (32-63 μm) and using preparative TLC. Infrared spectra were recorded on a Nicolet-Impact 410 spectrophotometer. ^1H and ^{13}C NMR were performed using Bruker Avance-III 400 and Bruker DRX-500 spectrometers and chemical shifts are reported in δ (ppm) relative to TMS (^1H) and CDCl_3 (^{13}C) as the internal standards. Melting points were determined on a Thomas Hoover Capillary Melting Point apparatus. High resolution mass spectra were recorded at the University of Arizona Mass Spectrometry facility using a JEOL HX110A mass spectrometer.

6.2 Chemical syntheses

6.2.1 6-endo-(Methylsulfinyl)bicyclo[2.2.1]heptan-2-endo-carboxylic acid pyrrolidine amide (3)

The method reported by Della and Graney for oxidizing thioethers to sulfoxides was slightly modified and used.⁴⁰ Sodium metaperiodate (93.9 mg, 0.439 mmol) was slowly added to a

solution of 6-*endo*-(methylthio)bicyclo[2.2.1]heptan-2- *endo*-pyrrolidine amide, **1** (100 mg, 0.418 mmol) in methanol/H₂O (0.75 mL/0.75 mL) cooled in ice-water bath under Ar. The mixture was allowed to warm up to room temperature and stirring continued for 3 h. The mixture was then filtered and the precipitate was washed with CH₂Cl₂ (5 mL) and the organic extracts concentrated under reduced pressure. Brine was added to the residue, extracted with CH₂Cl₂ (3 × 20 mL) and dried (anhydrous MgSO₄). The crude product was purified by silica gel chromatography eluting first with CH₂Cl₂ to remove impurities and then 5% methanol in CH₂Cl₂ to isolate sulfoxide **3** as a white solid (87%): mp ~130 °C (dec.); ¹H NMR (500 MHz, CDCl₃) δ 1.60 (m, 2H), 1.67 (ddd, *J* = 1.5, 5.5, 10.0Hz, 1H), 1.74 (ddd, *J* = 2.0, 5.0, 11.0Hz, 1H), 1.77-1.89 (m, 3H), 1.92-2.02 (m, 2H), 2.15 (m, 1H), 2.47 (t, *J* = 3.0Hz, 1H), 2.68 (s, 3H), 2.77-2.82 (m, 2H), 2.99 (m, 1H), 3.31-3.40 (m, 3H), 3.43-3.48 (m, 1H); ¹³C NMR (125 MHz, CDCl₃) δ 24.38, 26.78, 32.98, 34.09, 37.37, 40.30, 41.82, 43.17, 46.28, 46.84, 47.12, 67.52, 172.53; IR (KBr) 1020, 1432, 1624, 2873, 2926, 2965 cm⁻¹; HRMS (LCQ ESI⁺) *m/z* for C₁₃H₂₁NO₂S: Calcd. [M+H⁺], 256.1366; Found: 256.1365.

6.2.2 6-*endo*-(Methylsulfinyl)bicyclo[2.2.1]heptan-2-*exo*-carboxylic acid pyrrolidine amide (**10**)

Compound **10** was synthesized using same procedure for **3** to yield the desired product as slightly yellow oil (86%): ¹H NMR (600 MHz, CDCl₃) δ 0.81 (dd, *J* = 4.2, 10.8Hz, 0.46H), 0.89 (t, *J* = 7.8Hz, 0.10H), 1.21 (tt, *J* = 1.2, 7.2Hz, 0.60H), 1.30 (d, *J* = 10.2Hz, 0.64H), 1.40 (d, *J* = 10.8Hz, 0.67H), 1.68 (t, *J* = 10.8Hz, 0.70H), 1.73-1.95 (m, 7.55H), 2.21 (m, 0.34H), 2.40 (br s, 0.35H), 2.43 (br s, 0.64H), 2.48 (s, 1.92H), 2.51 (s, 1.00H), 2.72-2.78 (m, 1.27H), 2.81 (br s,

0.34H), 3.00 (dt, $J = 4.8, 11.4\text{Hz}$, 0.33H), 3.05-3.11 (m, 0.97H), 3.33-3.46 (m, 3.65H), 3.71 (dt, $J = 6.6, 10.2\text{Hz}$, 0.33H), 4.07 (q, $J = 6.6, 13.8\text{Hz}$, 0.31H); ^{13}C NMR (150 MHz, CDCl_3) δ 14.50, 24.54, 24.61, 26.43, 26.59, 27.39, 31.08, 33.78, 35.03, 36.50, 36.82, 37.06, 37.59, 38.69, 39.37, 39.44, 39.67, 42.98, 43.95, 46.19, 46.48, 46.62, 46.82, 60.66, 62.95, 66.00, 77.56, 172.78, 173.55; IR (KBr) 1036, 1339, 1436, 1636, 2873, 2959 cm^{-1} ; HRMS (LCQ ESI^+), m/z for $\text{C}_{13}\text{H}_{21}\text{NO}_2\text{S}$: Calcd. $[\text{M}+\text{H}^+]$, 256.1366; Found: 256.1365.

6.2.3. *endo*-Keto pyrrolidine **17** and the *exo*-keto pyrrolidine (**11**)

Treatment of a solution of the known ester **13**⁴⁵ in toluene with pyrrolidine under reflux afforded the keto *endo* pyrrolidine amide **18** in 34% yield. The crystalline compound was fully characterized spectroscopically and by high resolution mass spectrometry. Epimerization of the known *endo*-ester ketal **14**⁴⁴ with sodium methoxide afforded predominantly the *exo*-acid ketal **15** which was hydrolyzed quantitatively with aqueous hydrochloric acid to keto *exo* acid **16**. The structure of the keto *exo* acid (**16**) was established by IR, ^1H and ^{13}C NMR spectroscopic analyses. This intermediate was converted to keto *exo*-amide **11** by sequential reaction with oxalyl chloride followed by pyrrolidine. The structure of **11** was established by spectroscopic analysis and high resolution mass spectrometry.

6.2.4. *Bicyclo[2.2.1]heptan-2-one-6-endo-pyrrolidineamide (17)*

To a solution of methyl 6-oxobicyclo[2.2.1]heptane-2-carboxylate (**13**) (400 mg, 2.38 mmol, 1.0 eq) in dry toluene (2 mL) was added pyrrolidine (0.23 mL, 2.76 mmol, 1.2 eq) and the reaction mixture was heated at reflux under argon for 45 h. The solvent was removed under vacuum and

the residue was purified by preparative TLC (silica gel) using 4:6 chloroform-ethyl acetate mixture as eluent to give the pure product (**17**) as a pale yellow solid (168 mg, 34%). Further purification by crystallization from a mixture of DCM and hexane afforded the amide as colorless crystals: mp 82-83 °C; IR (thin film) 1747 (C=O, ketone), 1634 (C=O, amide) cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 3.52-3.43 (m, 4 H), 3.10 (dd, $J = 12.0, 7.8$ Hz, 1 H), 2.70 (d, $J = 3.7$ Hz, 2 H), 2.18 (dd, $J = 17.7, 4.1$ Hz, 1H), 2.07 (add, $J = 16.9, 3.9$ Hz, 1H), 2.00-1.92 (m, 4 H), 1.90-1.68 (m, 4H); ^{13}C NMR (100 MHz, CDCl_3) δ 215.2, 171.3, 53.3, 46.7, 46.2, 44.3, 43.0, 39.4, 35.1, 31.7, 26.3, 24.4; HRMS (TOF MS EI^+) calcd for $\text{C}_{12}\text{H}_{17}\text{NO}_2$: 207.1259, found 207.1249.

6.2.5 *endo*-6-Carbomethoxy-bicyclo[2.2.1]heptan-2-one-ethyleneketal (**14**)

A solution of the ester **13** (440 mg, 2.62 mmol, 1.0 eq) and ethylene glycol (0.3 mL, 5.24 mmol, 2.0 eq) in dry toluene (10 mL) containing p-TsOH (50 mg, 0.26 mmol, 0.1 eq) was heated at reflux overnight (Dean-Stark apparatus) under an argon atmosphere. The mixture was cooled, water (20 mL) added and extracted with ethyl acetate. The combined organic layers were washed with brine, dried (anhydr Na_2SO_4), filtered and concentrated on a rotary evaporator. The crude product was purified by flash silica gel column chromatography (hexane/ethyl acetate 85:15). The pure ketal ester **14** was obtained as colorless oil (380 mg, 68%): IR (neat) 1739 (C=O, ester) cm^{-1} ; ^1H NMR (500 MHz, CDCl_3) δ 3.95-3.71 (m, 4H), 3.68 (s, 3H), 2.74-2.69 (m, 1H), 2.64 (br d, $J = 4.2$ Hz, 1H), 2.30 (td, $J = 4.7, 1.4$ Hz, 1H), 1.92-1.86 (m, 2H), 1.81-1.75 (m, 1H), 1.69 (ddd, $J = 10.1, 4.1, 1.7$ Hz, 1H), 1.62 (dd, $J = 13.4, 3.5$ Hz, 1H), 1.44-1.40 (m, 1H); ^{13}C NMR (100 MHz, CDCl_3) δ 174.8, 115.5, 64.9, 64.1, 51.5, 47.3, 43.6, 42.4, 39.4, 35.3, 29.8.

6.2.6 *Bicyclo[2.2.1]heptan-2-one ethylene ketal exo-6-carboxylic acid (15)*

A solution of sodium methoxide in methanol was prepared by adding sodium metal (20 mg, 0.81 mmol, 1.5 eq) to dry methanol (2 mL). To this solution, endo ester **14** (115 mg, 0.54 mmol, 1.0 eq) in dry methanol (1 mL) was added. This solution was heated at reflux under argon overnight. The solution was then allowed to cool to room temperature and the solvent was removed under vacuum; the residue was acidified with ice-cold 3M HCl solution. The mixture was then extracted with ethyl ether, and the combined organic layers washed with brine, dried (anhydrous Na₂SO₄), filtered and concentrated to give pale yellow pasty solid. The crude product was purified by flash silica gel column chromatography (chloroform/ethyl acetate 90:10) to give the exo acid (**15**) as a white solid (68 mg, 60%): mp 94-95 °C; IR (KBr) 3400-2800 (OH, acid), 1695 cm⁻¹; ¹H NMR (400 MHz, CDCl₃), δ 10.0 (br s, 1H), 4.00-3.83 (m, 4H), 2.97 (dd, *J* = 9.4, 5.7 Hz, 1H) 2.47 (br s, 1H), 2.35 (t, *J* = 4.5 Hz, 1H), 1.94-1.81 (m, 2H), 1.67-1.61 (m, 2H), 1.51-1.47 (m, 2H); ¹³C NMR (100 MHz, CDCl₃) δ 182.0, 115.5, 64.8, 64.2, 47.9, 42.8, 40.0, 36.0, 35.5, 33.0. The deprotected acid **16** was also eluted from the column (chloroform/ethyl acetate 80:20) as pasty solid (9 mg, 10%).

6.2.7 *Bicyclo[2.2.1]hept-6-one-2-exo-carboxylic acid (16)*

Ketal acid **15** (74 mg, 0.37 mmol, 1.0 eq) was dissolved in a mixture of ether (4 mL) and 1N HCl solution (1.5 mL) with stirring. The reaction flask was stoppered and the mixture stirred at room temperature overnight. The layers were then separated and the ether layer was washed with brine, dried (anhydr Na₂SO₄), filtered and evaporated to give keto acid **16** as a white solid (56

mg, 98%). The acid was recrystallized from a mixture of dichloromethane and hexane to give colorless needle shaped crystals: mp 80-81 °C; IR (KBr) 3092-2560 (COOH), 1740 (C=O, ketone), 1700 (C=O, acid) cm^{-1} ; ^1H NMR (400 MHz, CDCl_3), δ 10.90 (br s, 1H), 2.93 (br d, J = 1.0 Hz, 1H), 2.78 (td, J = 4.3, 2.2 Hz, 1H), 2.71 (dd, J = 9.2, 4.9 Hz, 1H), 2.20-2.08 (m, 2H), 1.94-1.78 (m, 3H), 1.76-1.72 (m, 1H); ^{13}C NMR (100 MHz, CDCl_3) δ 215.5, 179.1, 53.5, 44.7, 40.5, 35.9, 35.3, 32.5.

6.2.8 *Bicyclo[2.2.1]heptan-2-one-6-exo-pyrrolidineamide (11)*

To a solution of bicyclo[2.2.1]heptane-6-one-2-exo-carboxylic acid, **16** (37 mg, 0.24 mmol, 1.0 eq) in dry benzene (0.5 mL) was added oxalyl chloride (0.03 mL, 0.36 mmol, 1.5 eq) and stirred at room temperature for 9 h. The benzene and excess oxalyl chloride were evaporated and the crude acid chloride was used for next step. The acid chloride was dissolved in dry THF (1 mL) and cooled to 0 °C under argon. Pyrrolidine (0.2 mL, 2.4 mmol, 10.0 eq) was added, stirred at 0 °C for 30 min and then warmed up to room temperature. After stirring at room temperature overnight, the solvent was removed under vacuum. To the residue water (10 mL) was added and extracted with ethyl acetate. The combined organic layers were washed with brine, dried (anhydr Na_2SO_4) and evaporated under vacuum to give the exo amide as yellow paste. The crude product was purified by flash silica gel column chromatography (chloroform/ethyl acetate 90:10) to give the pure exo amide (**11**) as a white solid (40 mg, 80%). Colorless crystals of **11** were grown from a mixture of ether and hexane: mp 79-80 °C; IR (thin film) 1742 (C=O, ketone), 1634 (C=O, amide) cm^{-1} ; ^1H NMR (400 MHz, CDCl_3), δ 3.52-3.39 (m, 4H), 2.76 (dt, J = 6.0, 3.1 Hz, 1H), 2.70 (bs, 1H), 2.65 (dd, J = 9.4, 5.1 Hz, 1H), 2.29-2.23 (m, 1H), 2.14- 2.07 (m, 2 H), 1.99-1.80

(m, 5H), 1.71-1.65 (m, 2H); ^{13}C NMR (100 MHz, CDCl_3) δ 217.0, 171.4, 54.1, 46.7, 46.3, 45.0, 39.7, 35.5, 35.4, 32.3, 26.3, 24.4; HRMS (TOF MS EI^+) calcd. for $\text{C}_{12}\text{H}_{17}\text{NO}_2$: 207.1259, found 207.1249.

6.3 X-ray Structure of Sulfoxide 3.

A colorless needle-like crystal was found to have one molecule in the asymmetric unit, and four molecules in the unit cell as a twinned crystal was collected at the University of California San Diego during the Bruker User's Meeting with the aid of Curtis Moore. The following programs were used in the data collection, structure solution and refinement: APEX2,⁶² SAINT,⁶³ SADABS,⁶⁴ SHELXTL,⁶⁵ and OLEX2.⁶⁶ The programs used for the molecular graphics were ORTEP⁶⁷ and MERCURY.⁶⁶ Table S1 contains the crystal data and structural refinement. Tables of the fractional atomic coordinates, anisotropic displacement parameters, bond lengths, bond angles and torsional angles are found in the Supporting Information (Tables S1-S7).

6.4 Metal-catalyzed oxidation of 1 and 2

Compounds **1** and **2** were subjected to Fenton oxidation. Stock solutions of **1** and **2** were prepared in ammonium acetate buffer (AAc, 20 mM, pH 6.9) at a concentration of 7 mM. H_2O_2 and Fe^{II} /EDTA solutions were freshly prepared at a concentration of 4 mM each in AAc buffer. All solutions were prepared under Ar. The final reaction mixture was prepared as follows: (i) AAc buffer (450 μL) was presaturated with Ar in sealed tubes, (ii) 50 μL of either the stock solution of **1** or **2** were mixed under Ar with 50 μL of Fe^{II} /EDTA in 7 mL AAc buffer, and the

reaction initiated by addition of 50 μL of the H_2O_2 stock solution. The reactions were run at 37°C in the dark for 5 to 60 min. Prior to opening the reactions to air, 100 μL of a DTPA solution (10 mM, Ar-saturated) were added to each sample.

6.5 Electrochemical Studies

The oxidation of compounds **1** and **2** was carried out in acetonitrile (MeCN) (extra dry, Acros) with 0.1 M NaClO_4 as supporting electrolyte. The reference electrode was 0.1 M Ag^+/Ag in MeCN and its potential was checked periodically against ferrocene (0.028 ± 0.002 V). Cyclic and differential pulse voltammetry was performed at Pt and glassy carbon electrodes, 2.0 and 3.0 mm, respectively. Controlled potential electrolyses employed a 7 mm glassy carbon rotating disk electrode rotating at 1,500 rpm.

6.6 Sample preparation for capillary LC-MS analysis

LC-MS analyses of the reaction mixtures were performed on a capillary Ultra Performance Liquid Chromatography (UPLC) System (Waters Corporation, Milford, MA, USA) at the University of Kansas. 10 μL of each sample were injected onto a C18 Vydac column (25 cm x 0.5 mm, 3.5 μm , Grace, Deerfield, IL), and eluted with a linear gradient delivered at the rate of 25 $\mu\text{L min}^{-1}$. Mobile phases consisted of water/acetonitrile/formic acid at a ratio of 99%, 1%, 0.08% (v:v:v) for solvent A and a ratio of 1%, 99%, 0.06% (v:v:v) for solvent B. The following linear gradient was set: 10-50% of solvent B within 20 min.

6.7. Nano-Electrospray Ionization Time-of-Flight MS (ESI-TOF-MS) and MS/MS analyses

ESI-MS and ESI-MS/MS spectra of **1**, **2**, and their oxidation products were acquired on a SYNAPT-G2 (Waters Corp., Milford, MA) at the University of Kansas. The SYNAPT-G2 instrument was operated for maximum resolution with all lenses optimized on the $[M + 2H]^{2+}$ ion from the [Glu]¹-fibrinopeptide B. The capillary voltage was set to 2.8 kV. The source and desolvation temperatures were set at 95°C and 190°C, respectively. The cone and desolvation gas flow were set to 20 L/hr and 200 L/hr, respectively. The cone voltage was 35 V and Ar was admitted to the collision cell. The spectra were acquired using a mass range of 50-2,000 amu (amu = atomic mass unit). The data were accumulated for 0.7 sec per cycle. MS/MS spectra were acquired by setting the MS¹ quadrupole to transmit a precursor mass window of ± 0.1 amu. The MS/MS survey continuum data were acquired using a scan time and an interscan time of 1.0 sec and 0.024 sec, respectively. A cone voltage ramp was set to 40-55 V.

6.8. Computational methods

Density Functional Theory (DFT) calculations were performed with the Gaussian-03 (G03) molecular orbital packages. The geometry optimization and frequency calculations were carried out at the B3LYP/6-31G(d) level by the hybrid HF-DFT procedure implemented in the Gaussian software package.⁶⁸ The geometries of the molecules were optimized in vacuum. The Gibbs free energies were calculated at 298 K (G_{298}^0) and are given in kcal/mol.

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7 Associated content

Supporting Information. CID of **1** and **2**; Proposed reaction pathways in fragmentation of **1** and **2**; NMR spectra of the synthetic compounds; ORTEP drawing and X-ray Crystallographic tables for sulfoxide **3**. **Supporting information for this article is available on the WWW under <http://dx.doi.org/10.1002/chem.2010xxxxx>.**

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