natural enantiomer of 10,10-difluoro-TXA_2 followed known methodology.

Conversion of (+)-**5** to the mesylate, mp 73°, $[\alpha]_D^{25}$ = +31.3° (*c* 0.55 in $CHCl_3$), 94%, with MsCl and pyridine followed by reaction with NaCN in DMF at 42 °C for 18 h yielded the nitrile (+)-**7**, $[\alpha]_D^{25}$ = +25.9° (*c* 0.96 in $CHCl_3$), which was reduced with DIBAL-H at −25 °C → +22 °C to the aldehyde **8**. Alternatively, the nitrile was hydrolyzed with 1 N NaOH at 42 °C for 1 h and methylated to the methyl ester (+)-**9**, $[\alpha]_D^{25}$ +16.8° (*c* 0.81 in $CHCl_3$), 81%, and the latter was reduced to the aldehyde **8** with Red-Al at −78 °C. Reduction of (+)-**9** with DIBAL-H at −20 °C gave the diol, which was converted to the diacetate (+)-**10** $[\alpha]_D^{25}$ = +29.1° (*c* 0.4).[8] Wittig reaction of **8** with the dianion of 4-carboxybutyltriphenylphosphonium bromide prepared with lithium hexamethyldisilazide in THF followed by methylation gave the cis and trans methyl esters **11** and **12** in 69% yield from **7**, in a 7:3 ratio. Separation was achieved by HPLC on silica gel using hexane/CH_2Cl_2/EtOAc in a 10:10:3 ratio. Swern oxidation of **11** [*m/z* 306 (M^+) 3.3%, 275 (M − CH_3O) 9%] gave the aldehyde **13**, which was converted with the anion of dimethyl 2-oxoheptylphosphonate to 10,10-difluoro-15-keto-TXA_2, **15**, $[\alpha]_D^{25}$ = +35° (*c* 0.2, $CHCl_3$). Similarly, the trans ester **12** afforded **16** via the aldehyde **14**. Diastereoselective reduction of **15** with the $LiAlH_4$ complex of (*S*)-(−)-1,1′-bi-2-naphthol[9] in THF yielded the methyl ester of 10,10-difluoro-TXA_2 **17**, $[\alpha]_D^{25}$ = +43.6° (*c* 0.05), *m/z* (TMS ether) 474.2617 (M^+) 8%, 443.2398 (M − OCH_3) 5%, 403.1713 (M − C_5H_{11}) 65%, 366.2656 ($C_{21}H_{38}O_3Si$) 19%, 225.1686 ($C_{13}H_{25}OSi$) 100%, 199.1501 ($C_{11}H_{23}OSi$) 35%, and its (*R*) isomer in a 9:1 ratio in 80% overall yield. Separation was achieved by HPLC on silica gel by using 1% *n*-propanol in hexane as the eluent.[10] A 1:1 mixture of **17** and **18** was obtained by reduction with $NaBH_4$ and $CeCl_3$. The corresponding trans isomers **19** and **20** were prepared from **16**. Hydrolysis of **17** with 0.5 N NaOH in 50% methanol/water afforded **1**: ^1H NMR ($CDCl_3$, 500 MHz) δ 5.87 (dd, 1 H, $J_{13,14}$ = 15.5 Hz, $J_{14,15}$ = 5.0 Hz, H-14), 5.77 (dd, 1 H, $J_{13,14}$ = 15.5 Hz, $J_{12,13}$ = 7.2 Hz, H-13), 5.64 (d, 1 H, $J_{9,11}$ = 4.5 Hz, H-11), 5.49 (dt, 1 H, $J_{5,6}$ = 10.3 Hz, $J_{6,7}$ = 6.5 Hz, H-6), 5.43 (dt, 1 H, $J_{5,6}$ = 10.3 Hz, $J_{4,5}$ = 6.5 Hz, H-5), 4.84 (dd, 1 H, $J_{H,F}$ = 8.6 Hz, $J_{9,11}$ = 4.5 Hz, H-9), 4.23 (q, 1 H, $J_{14,15}$ = $J_{15,16}$ = 5.0 Hz, H-15), 4.16 (t, 1 H, $J_{8,12}$ = $J_{12,13}$ = 7.2 Hz, H-12), 2.35 (m, 2 H, H-2), 2.23 (m, 3 H, H-4 and H-8), 2.05 (m, 2 H, H-7), 1.78 (m, 2 H, H-3), 1.60 (m, 2 H, H-16), 1.33 (m, 6 H, H-17, H-18, and H-19), 0.91 (t, 3 H, *J* = 6.5 Hz, H-20); ^{19}F NMR ($CDCl_3$, 376.2) Φ 110.07 (d, $J_{F,F}$ = 183.6 Hz), 138.30 (dd, $J_{F,F}$ = 183.6 Hz, $J_{H,F}$ = 8.5 Hz); *m/z* 370 (M^+ − H_2O); 299 (M^+ − H_2O − C_5H_{11}); 281 (M^+ − C_5H_{11} − $2H_2O$). Similarly, hydrolysis of **18**, **19**, and **20** yielded the corresponding free acids **21**, **22**, and **23**, respectively.

10,10-Difluoro-TXA_2 caused aggregation of washed human platelets at EC_{50} = 36 ± 3.6 nM indicating a potency 4.5 times greater than that reported for TXA_2.[11,12] Compound **1** stimulated contraction of canine saphenous veins with a potency (EC_{50} = 3.7 ± 0.8 nM) very similar to that reported for the TXA_2 mimic U46619.[13,14] In contrast, the isomeric compounds **21**, **22**, and **23** were antagonists of platelet aggregation stimulated by compound **1**. Compounds **21** and **22** were equipotent and approximately ten times more potent than **23**. On the other hand, compounds **21**, **22**, and **23** caused contraction of canine saphenous veins. All four compounds were capable of displacing the TXA_2 antagonist [^{125}I-PTA-OH[15] from its platelet binding site. Details of the bioassays will be reported elsewhere.[16]

The above results raise the possibility that platelet and vascular TXA_2 receptors are different. This class of compounds should prove to be useful tools to further explore these and other TXA_2 receptors. Moreover, the 7,7-difluoro-2,6-dioxo[1.1.3]bicycloheptane ring system, because of its close structural similarity to the TXA_2 nucleus and its stability during chemical reactions, presents unique opportunities for the construction of new molecules capable of binding to and interacting with TXA_2 receptors.

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Supplementary Material Available: Complete experimental data including spectroscopic data (^1H NMR and ^{19}F NMR) for all compounds (29 pages). Ordering information is given on any current masthead page.

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A Manganese(V)-Oxo Complex

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In metal-based oxidizing agents the metal center can have one of two roles: (i) in a *prima facie metallo-oxidant*, electron transfer from and/or atom transfer to or from the substrate is accompanied by a formal oxidation state change at the metal center;[2] (ii) in a *metallotemplate-oxidant* the metal center does not undergo a formal oxidation state change but activates the primary oxidant and/or substrate and/or arranges the primary oxidant and substrate in a favorable geometry for oxidation to proceed. For a number of years we have been working on perfecting ligand complements for prima facie metallo-oxidants.[3] We believe that the principal feature limiting the range of higher oxidation state middle and later transition-metal complexes is the rarity of strongly binding, oxidation resistant ligands. An important feature has

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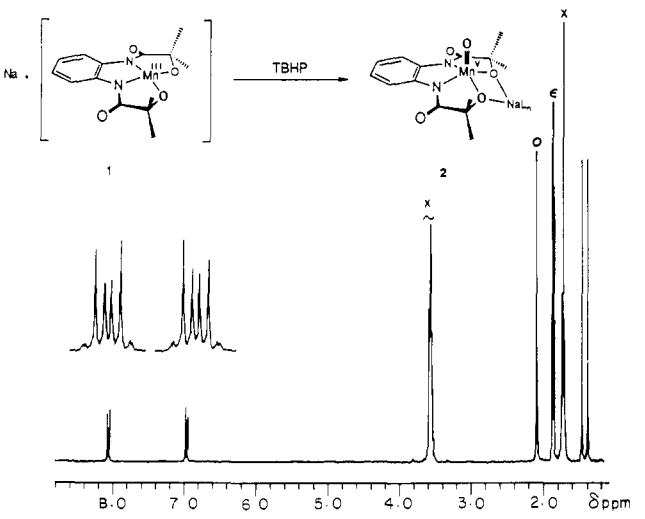

Figure 1. Reaction scheme and ^{1}H NMR (300 MHz, CD_3CN) of **2** (δ ppm aa'bb' 8.11, 2 H; 7.02, 2 H; 1.53, s, 6 H; 1.45, s, 6 H [X = THF signals, O = H_2O, ϵ = CD_2HCN]).

been to design ancilliary chelating ligand systems compatible with metal centers coordinated to the reduced species of molecular oxygen, especially superoxide, peroxide, and oxide. As one of the most significant challenges in this area, the production of oxo complexes for atom transfer oxidations is receiving considerable attention.[4]

Manganese, a central metal in oxidation chemistry, is now appearing in catalyzed oxygen atom transfers using porphyrin systems.[5] Manganese(V) porphyrin-oxo complexes are often proposed as intermediates in these processes, although the active intermediates are very reactive, and a representative complex has not yet been isolated. There is also considerable interest in the role of manganese-oxo complexes in photosynthetic oxygen evolution, and stable manganese(V)-oxo complexes should provide valuable benchmarks.[6] Here we report the synthesis and characterization of a stable manganese(V)-oxo complex of one of our ligand systems. To date, structural determinations of manganese-oxo complexes are limited to several polyoxo species of manganese(VII) and (VI).[4a,b]

The deep red Mn(III) complex, **1**, reacts with oxygen atom transfer oxidants to yield the deep green Mn(V)-oxo complex, **2** (Figure 1).[7] A useful synthesis proceeds as follows. A sample of powdered **1** (100 mg) was treated with enough *tert*-butyl hydroperoxide containing 5% *tert*-butyl alcohol and 5% water (Aldrich) to wet the powder (CAUTION: the oxidation is exothermic). Tetrahydrofuran (ca. 3 mL) was added after several minutes, and the green precipitate was collected and washed first with THF and then with diethyl ether to give the product in essentially quantitative yield. The ^{1}H NMR spectrum in CD_3CN shows that **2** is diamagnetic with two separate signals for the [η^4-HMPA-B]$^{4-}$ ligand methyl groups (Figure 1),[7] as would be expected for a d^2 square-pyramidal manganese(V) complex with a triply bonded axial oxo ligand.[8] Single crystals were grown by vapor diffusion of diethyl ether into an acetonitrile solution of **2** at room temperature, and the results of an X-ray crystal structure determination are shown in Figure 2.[9]

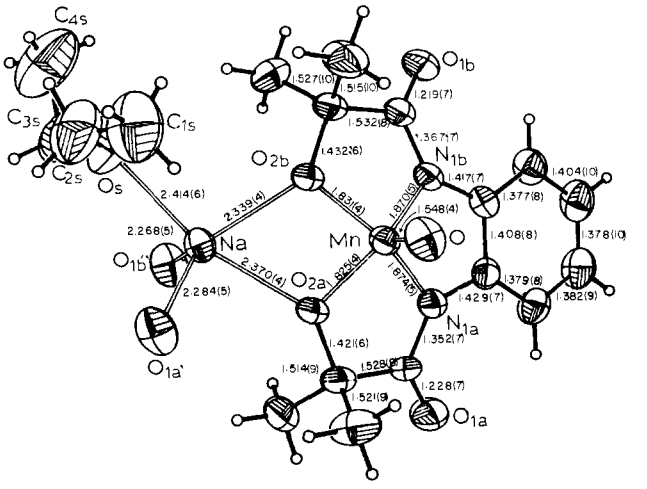

Figure 2. Molecular structure of **2**·Et_2O.

Several features of the structure of **2** are noteworthy. (a) The manganese-oxo bond (1.548 Å) is the shortest Mn–O bond to date[4a] and is consistent with a triple bond formulation (i.e., $Mn^-\equiv O:^+$). (b) The four donor atoms of the polyanionic chelating (PAC) ligand lie almost exactly in a plane (largest deviation 0.003 Å), and the manganese atom sits a large distance (0.62 Å) out of this plane as a secondary result of the strong bonding in the $Mn^-\equiv O:^+$ unit. (c) It is interesting that the amido-N ligands have remained nearly planar despite the high oxidation state of the metal.[10] We have shown that amido-N ligands coordinated to highly oxidizing metal centers can become stronger σ and/or π donors by distorting to distinctly nonplanar forms.[3d,11] (d) The bond distances in the benzene ring are consistent with an aromatic unit, and the bond distances from the ring to the amide nitrogen atoms are consistent with a single bond between the two sp^2 atoms.[3a,c,f,h,i] In the crystal structure of the tetramethylammonium analogue of the manganese(III) complex, **1**, the C_{ring}–N_{amide} bonds are shorter than in **2** (0.01–0.02 Å).[12] Thus other resonance structures[3i] involving electron transfer from the ring to the metal which shorten these bonds and reduce the formal oxidation state at the metal center can be ruled out. The oxidation is therefore metal-centered, and the formal oxidation state of the manganese center is V. In view of its formulation, compound **2** is remarkably stable under ambient conditions. We are presently investigating its reactivity.

Acknowledgment. We gratefully acknowledge Erich Uffelman for helpful discussions and the National Science Foundation (Grant No. CHE-8714720) for support.

Supplementary Material Available: A listing of atomic coordinates, anisotropic thermal parameters for non-hydrogen atoms,

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(8) The synthesis and structure of **1** and the infrared and Raman spectroscopic properties of **2** and related complexes will be the subject of forthcoming publications.

(9) Crystal data. The structure was solved by Crystalytics Company. Single crystals of **2**·OEt_2 are tetragonal at 20 ± 1°, space group $P4_3$–C^4_4 (no. 78) with a = 10.577 (2) Å, c = 19.2979 (4) Å, V = 21587 (8) Å^3 and Z = 4 [d_{calcd} = 1.366 g cm^{-3}; μ_a(Mo K$\bar{\alpha}$) = 0.64 mm^{-1}]. A total of 2558 independent reflections having 2θ < Mo K$\bar{\alpha}$ < 55.0° (the equivalent of 1.0 limiting Cu K$\bar{\alpha}$ spheres) were collected on a computer-controlled Nicolet autodiffractometer using full (0.90° wide) ω scans and graphite-monochromated Mo$\bar{\alpha}$ radiation. The structure was solved using a direct methods technique with the Nicolet SHELXTL software package as modified at Crystalytics Company. The resulting structural parameters have been refined to convergence [R_1-(unweighted based on F) = 0.038 for 1620 independent reflections having $2\theta_{MoK\bar{\alpha}}$ < 55.0° and $I > 3\sigma(I)$] by using counterweighted cascade block-diagonal least-squares techniques and a structural model which incorporated anisotropic thermal parameters for 28 non-hydrogen atoms and fixed isotropic thermal parameters for all hydrogen atoms. Methyl groups were refined as idealized sp^3-hybridized rigid rotors, and the remaining hydrogen atoms were fixed at idealized sp^2- or sp^3-hybridized positions with a C–H bond length of 0.96 Å.

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Conversion of a Protease into an Acyl Transferase: Selenolsubtilisin†

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Well-characterized proteins represent valuable starting points for the construction of new biocatalysts. For example, chemical methodology can be used to introduce non-natural amino acids or catalytic prosthetic groups into preexisting binding pockets to yield hybrid molecules that combine the intrinsic chemistry of the prosthetic group with the binding specificity of the protein template.[1] We are interested in the biological properties of selenium and have developed a chemical approach for converting reactive serine residues in proteins into selenocysteines. We report here the preparation and preliminary characterization of the first artificial selenoenzyme, selenolsubtilisin. Since the organic chemistry of selenium is extensive and of synthetic importance,[2] semisynthetic selenoenzymes with template-imposed selectivities might have considerable practical utility.

Subtilisin is a bacterial serine protease [EC 3.4.21.14] that is chemically[3] and structurally[4] well-studied. Conversion of the active site serine (Ser221) into selenocysteine was accomplished by a two-step protocol, analogous to Polgár's method for making thiolsubtilisin.[5] The side-chain alcohol of Ser221 of subtilisin Carlsberg (Sigma, Protease VIII) was selectively activated by reaction with phenylmethanesulfonyl fluoride (PMSF), and the sulfonylated enzyme (1 mM) was treated with a large excess of hydrogenselenide (ca. 0.5 M) in aqueous buffer (50 mM PIPES, 20 mM $CaCl_2$, pH 6.8) for 36 h at 40 °C. After gel filtration of the crude reaction mixture on Sephadex G-25, the protein fraction was reduced anaerobically with sodium borohydride, and selenolsubtilisin was purified by affinity chromatography on thiopropyl-Sepharose 6B. The yield of selenoenzyme was typically 40–50% based on phenylmethanesulfonylsubtilisin. Greater than 0.95 equivalents of selenium were incorporated per mol of subtilisin (ϵ^{280} 23 500 M^{-1} cm^{-1}) as judged by anaerobic titration of the reduced enzyme with 5,5′-dithiobis(2-nitrobenzoic acid)[6] and by neutron activation analysis.[7]

Serine (and cysteine) proteases cleave amides and esters by a two-step mechanism in which the active site nucleophile is transiently acylated by substrate. We wondered whether a selenol could assume the mechanistic role played by Ser221 in subtilisin. Like thiolsubtilisin,[8] selenolsubtilisin is a poor catalyst of amide hydrolysis, but it does promote the cleavage of activated acyl derivatives. For example, reduced selenolsubtilisin hydrolyzes

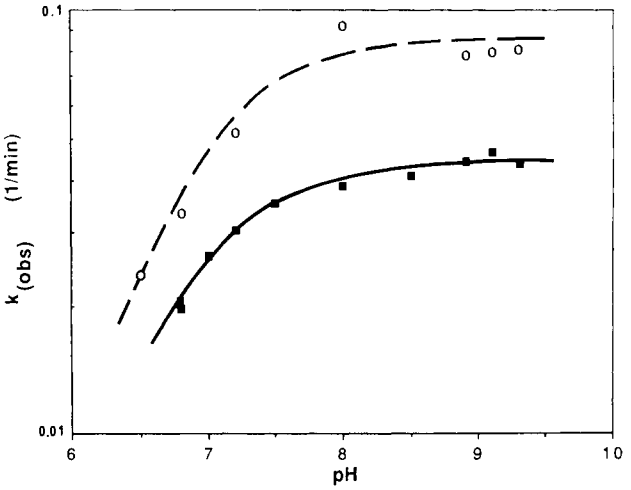

Figure 1. pH-rate profile for the hydrolysis of *S*-cinnamoylthiolsubtilisin (---) and *Se*-cinnamoylselenolsubtilisin (—) at 25.0 °C. The data were fit to the equation $k_{obsd} = (k_{lim})/(1 + [H^+]/K_a)$. The k_{lim} values for thiol- and selenolsubtilisin are 0.0837 and 0.0426 min^{-1}, respectively, and the relevant pK_a's are 6.9 and 6.8. Decinnamoylation of native subtilisin Carlsberg is described by a k_{lim} value of 22.8 min^{-1} and a pK_a of 7.7 (data not shown).

Table I. Ratio of Second-Order Rate Constants for Aminolysis and Hydrolysis of Cinnamoylated Subtilisin (O), Thiolsubtilisin, (S), and Selenolsubtilisin (Se)[a]

	$(k_{NH_2}/k_{OH})_O$	$(k_{NH_2}/k_{OH})_S$	$(k_{NH_2}/k_{OH})_{Se}$
n-BuNH_2	6	3600	81000
Gly-NH_2	19	7400	27000
NH_2OH	88	1400	7700

[a] Reactions were carried out in 0.1 M borate buffer at pH 9.3 and 25.0 °C, and decinnamoylation was monitored spectroscopically at 300 (O) and 320 nm (S and Se). Products were characterized by reversed-phase HPLC.

cinnamoylimidazole under anaerobic conditions via an acyl-enzyme adduct. The covalent intermediate (λ_{max} = 308 nm) was isolated by gel filtration at pH 5 and 4 °C. Deacylation of the enzyme was monitored spectroscopically at 25 °C as a function of pH. For comparison, Carlsberg *O*-cinnamoylsubtilisin (λ_{max} = 289 nm) and *S*-cinnamoylthiolsubtilisin (λ_{max} = 310 nm) were also prepared and studied.[9]

As shown in Figure 1, the pH-rate profile for hydrolysis of *Se*-cinnamoylselenolsubtilisin is very similar to that of *S*-cinnamoylthiolsubtilisin. Both curves can be fitted with a kinetic scheme in which optimal activity requires ionization of a group with pK_a of about 7. The titrating species is presumably His64 which is essential for activity in the native enzyme.[3] While the limiting rate constant is within a factor of 2 for thiol- and selenolsubtilisin, deacylation of *O*-cinnamoylsubtilisin is more than two orders of magnitude faster (k_{lim} = 23 min^{-1}). Since the alkaline hydrolysis of structurally analogous esters, thiol esters, and selenol esters is usually comparable, steric effects within the active site, rather than electronic factors, presumably account for this effect. Indeed, the covalent radius of oxygen (0.65 Å) is considerably smaller than that of sulfur (1.03 Å) or selenium (1.16 Å).[10] The properties of the selenium heteroatom thus make it a novel mechanistic probe of steric and electronic effects in enzymatic catalysis.

Selenol esters reportedly undergo aminolysis considerably faster than esters and thiol esters,[11] making them potentially useful for the *synthesis* of amide bonds.[12] We measured the partitioning

† Dedicated to the memory of E. T. Kaiser.

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