ments to form enol ether.[12] Two modes of fragmentation of the intermediate **5** have been considered. The first is a concerted cis elimination of the phosphine–tungsten complex from the intermediate **5** leading directly to **3** and **4a** (path a) in analogy with the direct formation of triphenylphosphine oxide in reactions of Wittig reagents with aldehydes. A second possible fragmentation pathway is an elimination from **5** giving vinyl ether, coordinately unsaturated $W(CO)_5$, and triphenylphosphine (path b). $W(CO)_5$ and PPh_3 could subsequently react to form **4a**. To distinguish between these two processes we have carried out the reaction of **1** and **2a** in the presence of tri-*p*-tolylphosphine which could compete with PPh_3 for capture of the coordinately unsaturated $W(CO)_5$ produced in pathway b. When equimolar amounts of **1** and $P(PhCH_3)_3$ were treated with an equivalent amount of **2a** in ether, a mixture of **4a** and pentacarbonyltri-*p*-tolylphosphinetungsten(0) (**4b**) was isolated in 42% yield by preparative thick-layer chromatography. Analysis of this mixture by nmr indicated a 1.8:1 ratio of **4a**:**4b**. In a control experiment it was demonstrated that $P(PhCH_3)_3$ does not react with **4a** under the reaction conditions. The greater amount of **4a** formed may be due to reaction of PPh_3 and $W(CO)_5$ within the initial solvent cage.

The reactions of metal–carbene complexes with other carbon nucleophiles are currently under investigation in an attempt to find synthetically useful ways of releasing the carbene ligand from metal–carbene complexes.

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(12) Reaction of isopropylidenetriphenylphosphorane with **1** did not lead to the formation of vinyl ethers. A possible explanation is that the increased steric crowding due to the additional methyl group in the isopropylidenephosphorane prevents the formation of the initial adduct **5**. The products of this reaction are currently under investigation.

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Active-Site Specific Inhibitors of Elastase

Sir:

Destruction of elastin and other fibrous connective tissue proteins associated with pulmonary emphysema and some inflammatory diseases has been shown to be caused by elastase and related neutral proteases.[1,2] Numerous site specific inhibitors of the homologous enzymes chymotrypsin and trypsin and for the related serine protease subtilisin BPN′ have been reported[3] and peptide chloromethyl ketones in particular have proven to be invaluable in the elucidation of the extended substrate binding sites of chymotrypsin and subtilisin by X-ray crystallography.[4] Although several stoichiometric inhibitors of elastase are known, reagents which resemble normal substrates have thus far proved inactive.[5,6] We wish to report the design and synthesis of a series of substrate-related peptide chloromethyl ketone inhibitors for the elastase from porcine pancreas, which has proven valuable in the study of the binding sites and biological function of elastolytic enzymes.

Benzyloxycarbonyl-L-alanyl chloromethyl ketone (Z-AlaCH$_2$Cl)[7] was prepared by reaction of an ether solution of Z-AlaCHN$_2$[8] with anhydrous HCl. Deblocking of Z-AlaCH$_2$Cl was accomplished at room temperature using HBr in acetic acid. The resultant low melting crystalline hydrobromide was coupled with a variety of blocked peptide acids using a mixed anhydride procedure[9] to obtain the compounds listed in Table I. The inhibitors were designed to partially resemble the normal elastase substrate elastin, a cross-linked polypeptide containing a high content of alanine, proline, and glycine, and to incorporate features found in the more reactive peptide substrates for this enzyme.[10]

Table I. Inhibition of Porcine Pancreatic Elastase (5×10^{-6} *M* with Peptide Chloromethyl Ketones in 5% Methanol at 30.0°

Inhibitor				pH	Inhibitor concn $\times 10^4$, *M*	k_{obsd}[a] $\times 10^3$, sec^{-1}	k_{obsd}/I,[c] M^{-1} sec^{-1}
P_4	P_3	P_2	P_1				
Ac-	Ala-	Gly-	AlaCH$_2$Cl	6.5	5.0	2.3[b]	0.47
Ac-	Ala-	Ala-	AlaCH$_2$Cl	6.5	5.0	13[c]	2.6
				5.0	5.0	2.6[d]	0.53
Z-	Gly-	Leu-	AlaCH$_2$Cl	5.0	5.0	5.0[b]	1.0
Ac-	Ala-	Ala-	AlaCH$_2$Cl	5.0	0.5	4.9[d]	9.8
Ac-	Ala-	Phe-	AlaCH$_2$Cl	5.0	0.5	4.2[d]	8.4
Ac-	Ala-	Pro-	AlaCH$_2$Cl	5.0	0.5	19[c]	38

[a] These values have a maximum spread of ±5%. [b] Average of two determinations. [c] Average of five determinations. [d] Average of three determinations.

Inhibition experiments were performed by adding a stock solution of elastase to a freshly prepared[11] solution of inhibitor in a 0.1 *M* acetate (pH 5.0) or 0.1 *M* phosphate (pH 6.5) buffer containing 10% (v/v) methanol. Residual enzyme activity was measured using a spectrophotometric assay for measuring the hydrolysis rate of BOC-Ala-ONP.[12] Good first-order kinetics were observed to at least 2 half-lives for all compounds. The data were processed using a least-squares computer

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program and correlation coefficients of greater than 0.995 were obtained. Results are listed in Table I.

Elastase activity depends upon a functional group with a pK of 6.5–6.85 (His-57)[13] and although the enzyme possesses only slight proteolytic activity at pH 5.0, most inhibition experiments were performed at this pH since the rates of reaction were too rapid to follow at higher pH values even when only a tenfold excess of inhibitor was employed. Our results clearly show that the rate of inhibition of elastase is markedly affected by the chain length of the chloromethyl ketone, Ac-Ala-Ala-Pro-AlaCH$_2$Cl being the most effective inhibitor. Inhibitors possessing a P_4 residue[14] are 8–34 times faster than those without. Simple substrate analogs such as Tos-AlaCH$_2$Cl and Tos-ValCH$_2$Cl are incapable of inhibiting the enzyme.[5,6] The rate of hydrolysis of simple peptide substrates by elastase is also markedly accelerated when the substrate contains a P_4 residue to interact with the S_4 binding subsite of the enzyme.[10] In contrast to the results obtained with substrates, where approximately a 25-fold rate acceleration is observed, a phenylalanyl residue in P_2 causes a slight decrease in the rate of inhibition. A leucyl or a prolyl residue in the P_2 position of an inhibitor accelerates the inhibition rate. An analogous effect with leucine has been observed in the rates of inhibition of the homologous serine protease chymotrypsin by a series of peptide chloromethyl ketones.[15] A more detailed discussion of the interactions between these peptide chloromethyl ketones and elastase must await the X-ray structure determination of an inhibited elastase derivative.

In addition to porcine elastase, several of the above compounds inhibit a human pancreatic enzyme which hydrolyzes BOC-Ala-ONP but not elastin.[16] The digestion of human lung tissue and rat aorta by pancreatic porcine elastase or by a human polymorphonuclear leukocyte elastase is completely inhibited by Ac-Ala-Ala-AlaCH$_2$Cl.[17] Also the increased agglutinability of mouse fibroblasts induced by treatment with a leukocyte lysosomal protease was inhibited by Ac-Ala-Ala-Pro-AlaCH$_2$Cl.[18]

The alanyl chloromethyl ketones thus far investigated appear to have a high specificity for elastolytic enzymes. Inhibition of trypsin by these compounds has not been observed. Several compounds (Ac-Ala-Gly-AlaCH$_2$Cl and Z-Gly-Leu-AlaCH$_2$Cl) inhibit bovine chymotrypsin, but the rates are less than 10% of those observed with elastase.[19] These inhibitors are therefore useful tools for studying the catalytic site, extended binding region, and biological function of elastase and related proteases.

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Stable Uranium(IV) Alkyl and Aryl Complexes

Sir:

Beginning with the synthesis of "uranocene,"[1] there has been a renaissance of interest in organoactinide chemistry.[2] Such questions as covalency, the involvement of 5f orbitals in chemical bonding, and chemical similarities to transition metals are of fundamental importance in actinide chemistry. To date, organouranium molecules are only known for polyhapto, π-bonding ligand systems, *viz.* h^5-C_5H_5 (cyclopentadienyl),[2] h^5-C_9H_7 (indenyl),[3] h^3-C_3H_5 (allyl),[2] h^8-C_8H_8 (cyclooctatetraene),[2,4] and h^6-C_6H_6 (arene),[5] with alkyl-substituted ligands included. No well-defined σ-bonded organometallics have yet been reported for uranium (or any actinide) in the chemical literature.[6] We report here the first synthesis of a series of such compounds, $(h^5\text{-}C_5H_5)_3UR$, and some of the more interesting properties of these new molecules.

The reaction of $(h^5\text{-}C_5H_5)_3UCl$[7] with organolithium or Grignard reagents produces the extremely air-sensitive alkyl and aryl compounds in high yield (60–80%).

$$(h^5\text{-}C_5H_5)_3UCl + RLi \longrightarrow (h^5\text{-}C_5H_5)_3U\text{-}R + LiCl$$

$$(h^5\text{-}C_5H_5)_3UCl + RMgX \longrightarrow (h^5\text{-}C_5H_5)_3U\text{-}R + MgXCl$$

Ia, R = CH_3
b, R = n-C_4H_9
c, R = allyl
Id, R = neopentyl
e, R = C_6F_5
f, R = i-C_3H_7

Purification can be achieved by hydrocarbon (toluene–hexane) extraction and crystallization (−78°).[8] Nmr

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