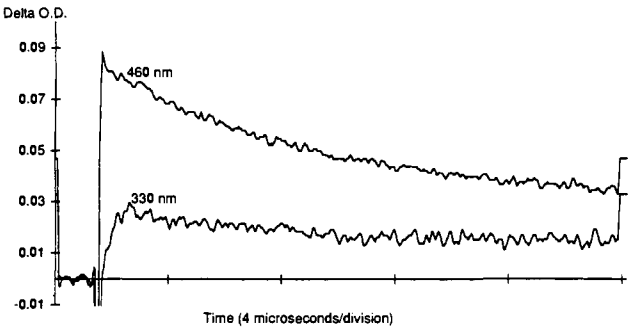
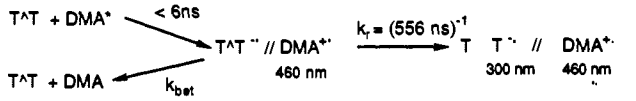


Figure 2. Kinetic traces from pulsed laser irradiation (308 nm, 6 ns, 50 mJ) of pH 12 thymine dimer/DMA solution. Absorbance change is monitored at 460 nm (DMA cation radical) and 330 nm (thymine anion radical).

$(k_q\tau\phi_r)^{-1}$.[11] The quantum efficiency of dimer anion radical cleavage, ϕ_r, is 0.4 at pH 12 and 0.1 at pH 7.



(2)

DMA cleaves thymine dimers via a reductive SET mechanism. This was determined by time-resolved laser spectroscopy. Pulsed laser photolysis[13] of DMA with thymine dimers at pH 12 gives the transient absorption spectra shown in Figure 1. Two bands appear: one at 460 nm, due to the cation radical of DMA,[14] and another at 300 nm, due to thymine monomer anion radical. The assignment of the latter is based on three considerations. First, this is very similar to absorption maxima for thymine monomer anion radicals reported by earlier works.[15] Second, when the substrate is changed to dimethylthymine dimers the low wavelength absorption band shifts to 330 nm. This demonstrates that low wavelength band is associated with the substrate rather than the sensitizer. Finally, we have independently generated the dimethylthymine monomer anion radical on our apparatus. Pulsed laser excitation of DMA in the presence of dimethylthymine gives a spectrum almost identical to the corresponding spectrum in Figure 1.

DMA cation radical appears within the 6 ns duration of the laser pulse, indicating that SET occurs on a time scale fast relative to the measurement. The band at 300 nm does not appear promptly after laser excitation, rather it grows in exponentially with a rate constant (fitted to first-order) of 4.6×10^6 s^{-1} (k_{obs}). The time profiles of both absorbance bands are shown in Figure 2. The observed rate constant for monomer anion growth, k_{obs}, is the sum of all rate constants which deplete the dimer anion radical ($k_{obs} = k_{bet} + k_r$).[16] The rate constant for the splitting step, k_r, is given as $k_r = k_{obs}\phi_r = 1.8 \times 10^6$ s^{-1}.[17]

In the absence of dimer, laser irradiation produces DMA cation radical and solvated electron (detected by its broad absorbance >600 nm). We considered that the 300 ns rise for the monomer anion radical might simply reflect the rate of attachment of the solvated electron to the thymine dimer. In this case, the solvated electron absorbance should have the same initial absorbance, but its decay rate should increase with added dimer. With added dimer, the initial absorbance at 600 nm is reduced to ca. 1/13 of its original intensity, indicating that dimer is interacting directly with DMA excited state. The fluorescence quenching experiment also demonstrates that the dimers are interacting directly with excited-state DMA and that solvated electron attachment is not a significant pathway.

For the reductive SET pathway to be operative, the pyrimidine dimer anion radicals must cleave rapidly enough to avoid nonproductive back electron transfer. The rate of back electron transfer in the enzymatic reaction is not known. However, the quantum yield for photorepair is ca. 0.7.[5d] This implies that the rate of back electron transfer is slower than cleavage. An upper limit for back electron transfer in the enzymatic reaction of $<10^6$ s^{-1} is predicted based on our data.[18] This is not unreasonable. Rates of SET are determined by properties of the external medium, the free energy change, distance between the donor and acceptor, and the relative orientation of the donor and acceptor.[19] The ordered environment of proteins can often hold the donor and acceptor at unfavorable distances and orientations.[20] Therefore, our results are entirely consistent with a reductive SET mechanism for DNA photorepair.[21]

Acknowledgment. We thank the donors of the Petroleum Research Fund, administered by the American Chemical Society, for support of this work.

(13) Excitation: 308 nm, 50 mJ, 6 ns. Sample solutions were purged with nitrogen and sealed in a 40-mL flow cell with quartz windows. Typical concentrations were 1.4×10^{-6} M sensitizer, 10 mM dimer, and 0.1 M phosphate buffer. To avoid complications due to proton transfer either to the dimer or monomer anion radicals the experiments were done at pH 12. Under these conditions the monomer anion radical is not protonated; see ref 15b. The role of proton transfer in the cleavage mechanism is currently under investigation in our laboratory and will be discussed in the full paper.

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(15) (a) Hayon, E. *J. Chem. Phys.* **1969**, *51*, 4881. (b) Deeble, D. J.; Das, S.; von Sonntag, C. *J. Phys. Chem.* **1985**, *89*, 5784.

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(18) This assumes that the enzyme does not actively promote the bond cleavage. It is also possible that the dimer cleavage occurs in a stepwise fashion whereby the 5-5 bond cleaves rapidly followed by rate-determining cleavage of the 6-6 bond. See: Witmer, M. R.; Altmann, E.; Young, H.; Begley, T. P.; Sancar, A. *J. Am. Chem. Soc.* **1989**, *111*, 9264.

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(20) Rates for exoergic SET in proteins in certain cases can be as low as 10^1 s^{-1}: Nocera, D. G.; Winkler, J. R.; Yocom, K. M.; Bordignon, E.; Gray, H. B. *J. Am. Chem. Soc.* **1984**, *106*, 5145.

(21) Recent picosecond measurements on the photolyase–dimer complex show that the flavin singlet is quenched with a rate constant of ca. 1×10^9 s^{-1}. Following this process a new species appears at 400 nm. Okamura, T.; Sancar, A.; Heelis, P. F.; Begley, T. P.; Hirata, Y.; Mataga, N. *J. Am. Chem. Soc.* **1991**, *113*, 3143.

A Convergent Enone Synthesis. Three-Component Coupling of Alkyl Iodides, Carbon Monoxide, and Allylstannanes by Free-Radical Carbonylation

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Free-radical carbonylation is now emerging as a new tool for the introduction of carbon monoxide into organic molecules, and we recently reported tin hydride mediated carbonylation of organic halides.[1] The tin hydride mediated system usually required moderate CO pressures (70–90 atm) and high-dilution conditions so as to cause the trapping of an alkyl radical by CO to predominate over the competing direct abstraction of a hydrogen atom from tributyltin hydride by the alkyl radical. In principle, if a competing reaction is much slower than the trapping of the alkyl

(1) (a) Ryu, I.; Kusano, K.; Ogawa, A.; Kambe, N.; Sonoda, N. *J. Am. Chem. Soc.* **1990**, *112*, 1295. (b) Ryu, I.; Kusano, K.; Masumi, N.; Yamazaki, H.; Ogawa, A.; Sonoda, N. *Tetrahedron Lett.* **1990**, *31*, 6887. (c) Ryu, I.; Kusano, K.; Hasegawa, M.; Kambe, N.; Sonoda, N. *J. Chem. Soc., Chem. Commun.* **1991**, 1018. (d) Ryu, I.; Kusano, K.; Yamazaki, H.; Sonoda, N. *J. Org. Chem.* **1991**, *56*, 5003.

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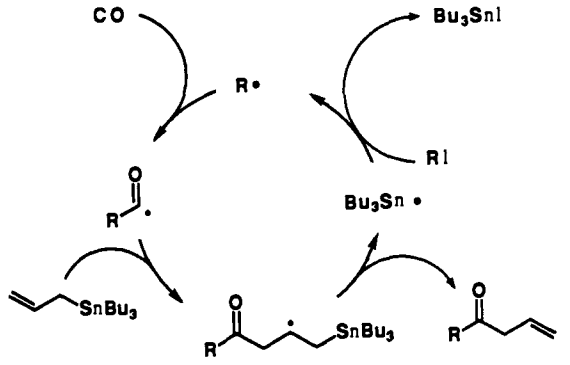
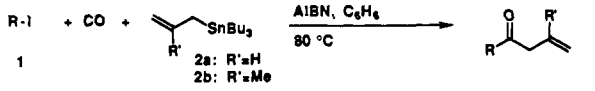
Scheme I



radical by CO, free-radical carbonylation under much lower CO pressure should be achieved. Now we show that this idea can be realized by use of allylstannane as a radical mediator. We report here the three-component coupling reaction of alkyl iodides, carbon monoxide, and allylstannanes by free-radical carbonylation, which leads to a convergent synthesis of β,γ-unsaturated ketones (eq 1).



Free-radical chain addition of alkyl radicals to allylstannanes to give allylated alkanes[2] is known to occur at a relatively slow rate compared with hydrogen abstraction from tin hydride.[3] Thus, we believed that three-component coupling of alkyl halides, CO, and allylstannanes by a free-radical chain process would be achieved under conditions of low CO pressure. AIBN-induced radical reaction of octyl iodide (**1a**) with allylstannanes (**2a** and **2b**) under CO pressure was pursued in detail by changing the reaction conditions. Interestingly, *free-radical carbonylation of a primary alkyl iodide* **1a** *to give* **3** and **4** *proceeded smoothly even under very low CO pressures (2–10 atm)* (Table I, runs 1–4). The mechanism of the reaction involves (1) the trapping of an alkyl radical by CO to give an acyl radical, (2) the addition of the acyl radical to an allylstannane, and (3) the elimination of stannyl radical to give β,γ-unsaturated ketones (Scheme I).

Some other results for the synthesis of β,γ-unsaturated ketones are also summarized in Table I. All of the products were isolated by flash chromatography on silica gel.[4] This new method for synthesizing enones can work similarly with secondary and tertiary alkyl iodides (runs 5–8).[5] In these cases, the employment of enhanced CO pressure gave better selectivity of carbonylated products relative to allylated alkanes.[6] With 3-hexenyl iodides **1e** and **1f**, *E*-form ketone **8** formed mainly, irrespective of the stereochemistry of the starting substrates (runs 9 and 10). This may be a result of an equilibrium between (*E*)- and (*Z*)-3-hexenyl radicals via cyclopropylmethyl radical.[7] Alkyl iodide **1g** possessing β-ethoxycarbonyl functionality could be also carbonylated to give 4-oxo ester **9** possessing β,γ-unsaturation (run 11). The case of carbonylative cyclization with 4-hexenyl iodide (**1h**) is noteworthy, since the result well features the characteristics of this system. The major product obtained was six-membered-ring δ,ϵ-unsaturated ketone **11** rather than five-membered-ring ketone **10** (run 12). This can be explained as a result of the isomerization of kinetically favored (2-oxocyclopentyl)ethyl radical **13**[8] to thermodynamically favored **14**, which is permitted by the slow reaction of alkyl radical **13** with allylstannane. On the other hand, in the case of 5-hexenyl iodide (**1i**), cyclopentylmethyl ketone **12** was obtained, suggesting that *radical clock* cyclization of 5-hexenyl radical **15** took place at a more rapid rate than the trapping of **15** by CO (run 13).

Table I. A Convergent Synthesis of β,γ-Enones by Three-Component Coupling Reactions of Alkyl Iodides, Carbon Monoxide, and Allylstannanes[a]

run	R-I	allyl-Sn	conditions	product	yield[b]
1	1a	2a	[R-I]=0.1 M, CO, 10 atm	3	73 %
2	1a	2b	[R-I]=0.1 M, CO, 10 atm	4	74 %
3	1a	2b	[R-I]=0.1 M, CO, 5 atm	4	69 %
4	1a	2b	[R-I]=0.05 M, CO, 2 atm	4	58 %
5	1b	2a	[R-I]=0.1 M, CO, 10 atm	5	70 %
6	1c	2a	[R-I]=0.1 M, CO, 30 atm	6	62 %
7	1c	2a	[R-I]=0.1 M, CO, 10 atm	6	45 %
8	1d	2a	[R-I]=0.1 M, CO, 30 atm	7	60 %
9	1e	2b	[R-I]=0.1 M, CO, 10 atm	8	71 % (E/Z = 88/12)[c]
10	1f	2b	[R-I]=0.1 M, CO, 10 atm	8	68 % (E/Z = 75/25)[c]
11	1g	2b	[R-I]=0.05 M, CO, 10 atm	9	65 %
12	1h	2b	[R-I]=0.1 M, CO, 10 atm	10 (6 : 94)[c] 11[d]	64 %
13	1i	2b	[R-I]=0.1 M, CO, 10 atm	12	66 %

[a] Conditions: **1** (1 mmol), **2** (2 mmol), AIBN (0.2–0.4 mmol), C_6H_6 (10 or 20 mL), CO (2–30 atm), 80 °C, 12 h. For a typical procedure, see footnote 4. [b] Refers to yields of purified materials. [c] Determined by GLC. [d] **11** was obtained as a 10:90 mixture of cis and trans isomers.

(2) (a) Kosugi, M.; Kurino, K.; Migita, T. *J. Organomet. Chem.* **1973**, *56*, C11. (b) Grignon, J.; Pereyre, M. *J. Organomet. Chem.* **1973**, *61*, C33. (c) Grignon, J.; Servens, C.; Pereyre, M. *J. Organomet. Chem.* **1975**, *96*, 225. (d) Migita, T.; Nagai, K.; Kosugi, M. *Bull. Chem. Soc. Jpn.* **1983**, *56*, 2480. (e) Keck, G. E.; Yates, J. B. *J. Am. Chem. Soc.* **1982**, *104*, 5829. (f) Keck, G. E.; Enholm, E. J.; Yates, J. B.; Wiley, M. R. *Tetrahedron* **1985**, *41*, 4079. (g) Giese, B.; Linker, T.; Muhn, R. *Tetrahedron* **1989**, *45*, 935. (h) Baldwin, J. E.; Adlington, R. M.; Mitchell, M. B.; Robertson, J. *J. Chem. Soc., Chem. Commun.* **1990**, 1574. (i) Toru, T.; Okumura, T.; Ueno, Y. *J. Org. Chem.* **1990**, *55*, 1277. (j) Mizuno, K.; Ikeda, M.; Toda, S.; Otsuji, Y. *J. Am. Chem. Soc.* **1988**, *110*, 1288. (k) Curran, D. P.; van Elburg, P. A. *Tetrahedron Lett.* **1989**, *30*, 2501. (l) Curran, D. P.; Shen, W.; Zhang, J.; Heffner, T. A. *J. Am. Chem. Soc.* **1990**, *112*, 6738.

(3) Recently Curran and Giese et al. have estimated its brief rate constant to be 10^4–10^5 M^{-1} s^{-1} at 50–80 °C, which is significantly slower than H abstraction from *n*-Bu_3SnH. See: Curran, D. P.; van Elburg, P. A.; Giese, B.; Gilges, S. *Tetrahedron Lett.* **1990**, *31*, 2861.

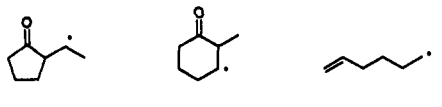
(4) Typical procedure: A mixture of benzene (10 mL), octyl iodide (**1a**; 239 mg, 1.0 mmol), allyltributylstannane (**2a**; 660 mg, 1.99 mmol), and AIBN (166 mg, 0.4 mmol) was placed in a 50-mL stainless steel autoclave equipped with a glass tube inserted. The autoclave was then pressurized with 10 atm of CO and was heated, with stirring, at 80 °C for 12 h. After excess CO was discharged, the benzene was evaporated. The residue was dissolved in Et_2O (10 mL). The ethereal solution was treated with saturated aqueous KF. The *n*-Bu_3SnF that was precipitated was removed by vacuum filtration. The solid was washed with Et_2O (3 × 10 mL). The two liquid layers were separated. The organic layer was dried ($MgSO_4$) and concentrated. The residue was purified by flash chromatography on silica gel (Et_2O/hexane, 1:9). The major fraction eluted from the column contained 132.1 mg (73%) of octyl propenyl ketone (**3**).

(5) Recent synthesis of β,γ-unsaturated ketones, see: (a) Trost, B. M.; Dyker, G.; Kulawiec, R. J. *J. Am. Chem. Soc.* **1990**, *112*, 7809. (b) Yano, K.; Baba, A.; Matsuda, H. *Chem. Lett.* **1991**, 1181.

(6) The marked efficiency in the carbonylation of a primary alkyl radical may be taken as a reflection of the slow back-reaction (decarbonylation). For kinetic data of decarbonylation, see: (a) Lunazzi, L.; Ingold, K. U.; Scaiano, J. C. *J. Phys. Chem.* **1983**, *87*, 529. (b) Neville, A. G.; Brown, C. E.; Rayner, D. M.; Lusztyk, J.; Ingold, K. U. *J. Am. Chem. Soc.* **1991**, *113*, 1869. (c) Lipscher, J.; Fischer, H. *J. Phys. Chem.* **1984**, *88*, 2555. (d) Fischer, H.; Paul, H. *Acc. Chem. Res.* **1987**, *20*, 200 and references therein.

(7) Beckwith, A. L. J.; Bowry, V. W. *J. Org. Chem.* **1989**, *54*, 2681. Cf. ref 1a.

(8) Lusztyk, J.; Lusztyk, E.; Maillard, B.; Ingold, K. U. *J. Am. Chem. Soc.* **1984**, *106*, 2923. Cf.: Free-radical carbonylation of **1h** with tin hydride gave a 67:33 mixture of 2-ethylcyclopentanone and 3-methylcyclohexanone.[1c]



13 14 15

Thus, we have demonstrated a convergent synthesis of β,γ-unsaturated ketones by free-radical-mediated three-component coupling of alkyl iodides, CO, and allylstannanes. The results above show that *free-radical carbonylation of an alkyl radical under low CO pressure is possible when the competing reaction of the alkyl radical is sluggish.* The success of low-pressure carbonylation now encourages us to move on the second stage of this work, other multicomponent coupling processes, double CO trapping with cyclizations, etc. The full scope of this methodology is under active study in our laboratory.

Supplementary Material Available: Detailed experimental procedures and characterization of products (4 pages). Ordering information is given on any current masthead page.

Mechanism-Based Inactivation of a Bacterial Phosphotriesterase by an Alkynyl Phosphate Ester

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Enzyme-catalyzed reactions involving the transfer of phosphoryl groups make up a large class of group-transfer reactions that are central to metabolism. These enzymes catalyze reactions that involve nucleophilic attack at an electrophilic phosphorus center, with subsequent cleavage of a phosphorus–oxygen or phosphorus–nitrogen bond. Although phosphoryl-transfer enzymes utilize a variety of mechanistic alternatives, relatively few suicide substrates have been designed or discovered that react with this class of enzymes. Recently, a series of alkynyl phosphate esters have been synthesized,[1] and these compounds have the potential to form a highly reactive ketene intermediate upon cleavage of the phosphorus–oxygen bond as illustrated in Scheme I.

The inherent reactivity of the ketene intermediate is expected to lead to the rapid and irreversible inactivation of enzyme activity if a nucleophilic group of an amino acid side chain is appropriately situated within the active site. On the basis of the previously determined substrate specificity for the phosphotriesterase from *Pseudomonas diminuta*, the alkynyl phosphate esters would be expected to be efficiently hydrolyzed by this enzyme.[2] In this report we demonstrate that this novel class of enzyme inhibitor rapidly inactivates the bacterial phosphotriesterase via a mechanism-based process.

The bacterial phosphotriesterase used in this study was purified to apparent homogeneity using the method of Dumas et al.[2] The diethyl 1-hexynyl phosphate was synthesized according to the method of Stang et al.[1] The inhibition experiments with the alkynyl phosphate ester were conducted in 3% acetonitrile containing 100 mM PIPES,[3] pH 7.0, at 25 °C.

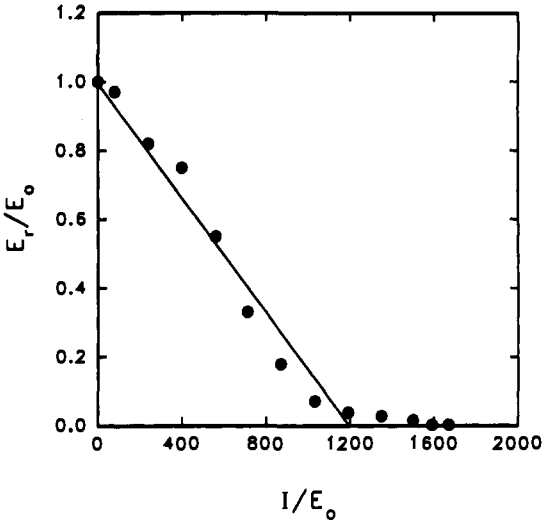
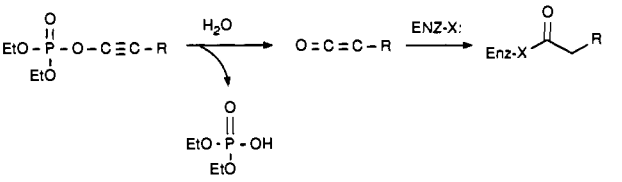
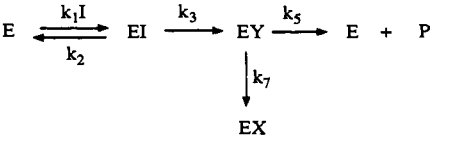


Figure 1. Inactivation of phosphotriesterase (125 pM) by variable amounts of diethyl 1-hexynyl phosphate. Enzyme and inhibitor were mixed at pH 7.0, and the remaining enzyme activity was measured after 15 min of incubation. Additional details are given in the text.

Scheme I



Scheme II



The initial inhibition experiments with diethyl 1-hexynyl phosphate and the phosphotriesterase were conducted at a constant enzyme concentration of 50 pM. Incubation of the inhibitor (1–1000 μM) and enzyme for 2 min at pH 7 resulted in >99% loss of all enzyme activity. Dialysis of the inhibited enzyme solution against 100 mM PIPES, pH 7.0, for up to 72 h gave no detectable increase in enzyme activity. In a control experiment, uninhibited enzyme, dialyzed against 100 mM PIPES, pH 7.0, for 72 h lost only 5% of the original activity. The failure of extensive dialysis to reactivate the enzyme is consistent with the formation of a covalent bond between enzyme and inhibitor. Incubation of the inhibited enzyme with 50–100 mM hydroxylamine, at pH 7.0, for up to 24 h also failed to regenerate any enzyme activity.

The efficiency of enzyme inactivation by the alkynyl phosphate ester was determined by incubation of a fixed enzyme concentration (125 pM) with variable concentrations of inhibitor (0–0.21 μM) as described by Knight and Waley.[4] Shown in Figure 1 is the plot of the fraction of enzyme activity remaining ($[E]_r/[E]_0$) versus the initial ratio of inhibitor to enzyme ($[I]/[E]_0$). The intercept on the horizontal axis is 1200, and thus 1200 ester molecules are hydrolyzed for every enzyme molecule inactivated. The inactivation of the phosphotriesterase by the alkynyl phosphate ester is therefore consistent with the model illustrated in Scheme II, where E is the enzyme, I is the inhibitor, EI is the initial noncovalent enzyme–inhibitor complex, and EY is the activated species which can either react within the complex to produce the

† This paper is dedicated to the memory of Jeffrey N. Blankenship, who died on June 16, 1991.

(1) Stang, P. J.; Boehshar, M.; Lin, J. *J. Am. Chem. Soc.* **1986**, *108*, 7832. Stang, P. J.; Kitamura, T.; Boehshar, M.; Wingert, H. *J. Am. Chem. Soc.* **1989**, *111*, 2225.

(2) Dumas, D. P.; Caldwell, S. R.; Wild, J. R.; Raushel, F. M. *J. Biol. Chem.* **1989**, *264*, 19659.

(3) PIPES is an abbreviation for piperazine-*N*,*N*′-bis(2-ethanesulfonic acid).

(4) Knight, G. C.; Waley, S. G. *Biochem. J.* **1985**, *225*, 435.

0002-7863/91/1513-8560$02.50/0