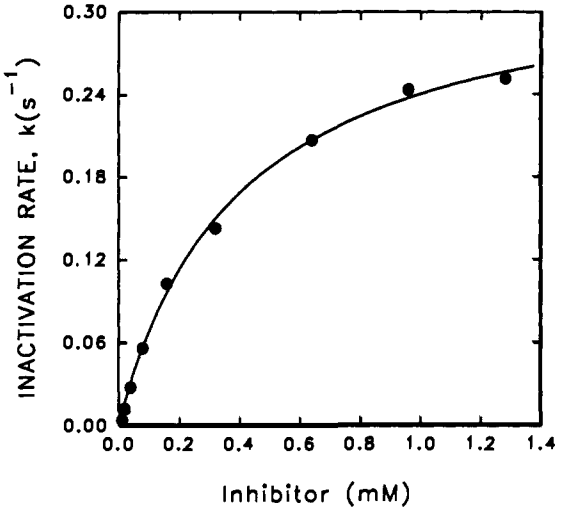

Figure 2. Variation of inactivation rate versus the initial concentration of diethyl 1-hexynyl phosphate. The solid line represents a fit of the data to eq 1. Additional details are given in the text.

irreversibly inhibited enzyme (EX) or dissociate to yield free enzyme (E) and product (P). The value of k_5/k_7 is the partition ratio of 1200. The products of the hydrolysis reaction were identified by ^1H and ^{31}P NMR spectroscopy to be diethyl phosphate and hexanoic acid.

Attempts to directly measure the rate of inactivation by the alkynyl phosphate ester failed because incubation of the enzyme and inhibitor eliminated all catalytic activity within 1 min and thus no kinetic data were obtainable. This problem was circumvented by the rapid mixing of enzyme and inhibitor with a High-Tech stopped-flow spectrophotometer (Model SF-51). The chromogenic substrate paraoxon (diethyl *p*-nitrophenyl phosphate) was added as a reporter substrate to monitor the activity of the enzyme as a function of time. For these experiments the ratio of the initial inhibitor concentration ([I]) to the initial enzyme concentration ($[E]_0$) was kept constant at 1600. Incubation of enzyme, inhibitor, and 0.25 mM paraoxon resulted in time-dependent loss of catalytic activity when the reaction was continuously monitored at 400 nm. Shown in Figure 2 is the plot of the apparent first-order rate constant for enzyme inactivation versus the initial concentration of inhibitor at constant $[I]/[E]_0$. These data were fitted to eq 1, where k_{obsd} is the observed first-order

$$k_{obsd} = (k_{in}[I])/(K_i(1 + A/K_a) + [I]) \qquad (1)$$

rate constant for inactivation, k_{in} is the maximal rate of inactivation at saturating inhibitor, [I] is the initial inhibitor concentration, K_i is the Michaelis constant for [I], A is the paraoxon concentration, and K_a is the Michaelis constant for A. The maximal rate of inactivation at saturating inhibitor is 0.33 ± 0.01 s^{-1} and $K_i = 58 \pm 4$ μM (A = 0.25 mM and K_a = 42 μM).

Using the minimal model for mechanism-based inhibition which appears in Scheme II, the rate expression for the inactivation rate is $k_{in} = (k_3k_7)/(k_3 + k_5 + k_7)$. Since $k_5 \gg k_7$, the minimum value of the rate constant (k_7) for the reaction of the putative ketene intermediate with the protein is 0.33 s^{-1}. The minimum value of the rate constant for P–O bond cleavage and product release (k_3 and k_5) is 410 s^{-1}. This value compares very favorably with the k_{cat} for paraoxon of 2100 s^{-1}, and thus the alkynyl phosphate esters are quite respectable substrates for the bacterial phosphotriesterase.

Although irreversible inhibition of many enzymes by alkynes and alkenes has been previously reported,[5] diethyl 1-hexynyl phosphate is the first alkynyl phosphate ester to irreversibly inactivate an enzyme. This compound is the first mechanism-based inhibitor reported for the bacterial phosphotriesterase. The alkynyl phosphate esters may prove to be useful for the active-site labeling of the bacterial phosphotriesterase.

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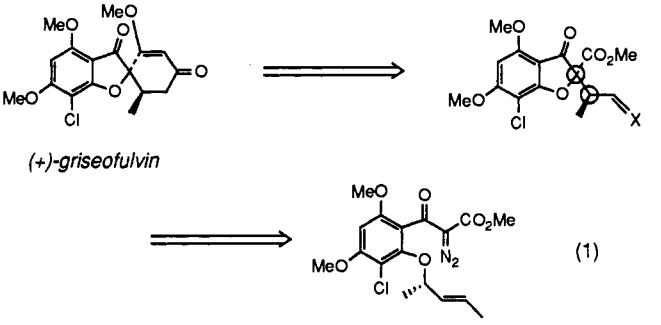
(5) Abeles, R. H. In *Enzyme-Activated Irreversible Inhibitors*; Seiler, N., Jung, M. J., Koch-Weser, J., Eds.; Elsevier/North-Holland Biochemical Press: Amsterdam, 1978.

Total Synthesis of (+)-Griseofulvin

Michael C. Pirrung,* William L. Brown, Sushil Rege, and Pierre Laughton

Department of Chemistry, Duke University
P. M. Gross Chemical Laboratory
Durham, North Carolina 27706
Received July 18, 1991

Griseofulvin is a classical antifungal agent still used in the treatment of dermatomycoses in animals and humans[1] and in plant protection. It acts via binding specifically to fungal tubulin at submillimolar concentrations[2] and has been used in cell biological studies to induce the formation of large (up to 100 nm diameter), hollow tubes called megatubules.[3] It has been prepared by several routes, as part of structure–activity studies[4] and in the development of modern organic synthesis.[5] However, all of the syntheses reported thus far yield the racemate. The spirocyclic structure and vicinal stereogenic centers (eq 1) of griseofulvin form a challenging proving ground for asymmetric synthesis methods. This communication reports the first synthesis of the natural enantiomer of griseofulvin and demonstrates the power of the sigmatropic rearrangement of oxonium ylides[6] in diastereoselective and enantioselective synthesis.



(1)

Commercially available dimethoxyphenol is chlorinated by a known method[7] and acetylated to provide **2** (Scheme I). Fries rearrangement gives an *o*-hydroxyacetophenone, which is used directly in a Mitsunobu coupling[8] with (*R*)-pent-3-en-2-ol of 97%

(1) Davies, R. R. *Antifungal Chemotherapy*; Wiley & Sons: New York, 1980; p 149.
(2) Starling, D. *J. Cell Sci.* **1976**, *20*, 91. Kuriyama, R.; Borisy, G. G.; Binder, L. I.; Gottesman, M. M. *Exp. Cell. Res.* **1985**, *160*, 527. Roobol, A.; Gull, K.; Pogson, C. I. *Biochem. J.* **1977**, *167*, 39. Weber, K.; Wehland, J.; Herzog, W. *J. Mol. Biol.* **1976**, *102*, 817; **1977**, *111*, 329. Sloboda, R. D.; Van Blaricom, G.; Creasey, W. A.; Rosenbaum, J. L.; Malawista, S. E. *Biochem. Biophys. Res. Commun.* **1982**, *105*, 882.
(3) Hebert, C. D.; Steffers, W.; Wille, J. J., Jr. *Exp. Cell Res.* **1980**, *126*, 1.
(4) Mir, L.; Oustrin, M.-L.; Lecointe, P.; Wright, M. *FEBS Lett.* **1978**, *88*, 259. MacMillan, J. *J. Chem. Soc.* **1959**, 1823. Arkley, V.; Attenburrow, J.; Gregory, G. I.; Walker, T. *Ibid.* **1962**, 1260, 1269, 1277. Tomozane, H.; Takeuchi, Y.; Choshi, T.; Kishida, S.; Yamato, M. *Chem. Pharm. Bull.* **1990**, *38*, 925. Yamato, M.; Takeuchi, Y.; Tomozane, H. *Synthesis* **1990**, 569.
(5) Stork, G.; Tomasz, M. *J. Am. Chem. Soc.* **1978**, *100*, 315. Danishefsky, S.; Walker, F. J. *Ibid.* **1979**, *101*, 7018. Taub, D.; Kuo, C. H.; Slates, H. L.; Wendler, N. L. *Tetrahedron* **1963**, *19*, 1. Day, A. L.; Nabney, J.; Scott, A. I. *J. Chem. Soc.* **1961**, 4067. Brossi, A.; Baumann, M.; Gerecke, M.; Kyburz, E. *Helv. Chim. Acta* **1960**, *43*, 207.
(6) Pirrung, M. C.; Werner, J. A. *J. Am. Chem. Soc.* **1986**, *108*, 6060. Johnson, C. R.; Roskamp, E. J. *J. Am. Chem. Soc.* **1986**, *108*, 6062. Kirmse, W.; Kund, D. *J. Am. Chem. Soc.* **1989**, *111*, 1465. Doyle, M. P.; Bagheri, V.; Claxton, E. E. *J. Chem. Soc., Chem. Commun.* **1990**, 46. Doyle, M. P.; Bagheri, V.; Harn, N. K. *Tetrahedron Lett.* **1988**, *29*, 5119.
(7) Stork, G.; Tomasz, M. *J. Am. Chem. Soc.* **1964**, *86*, 471.

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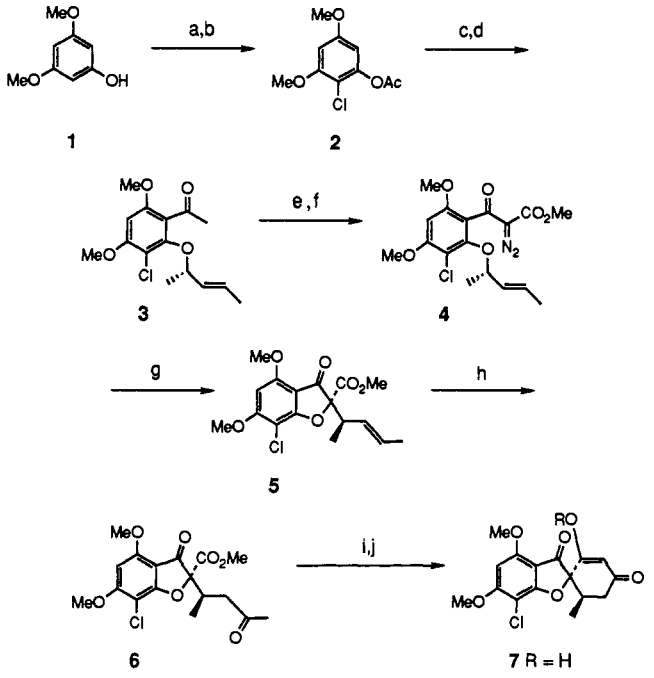
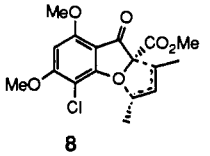
Scheme I[a]



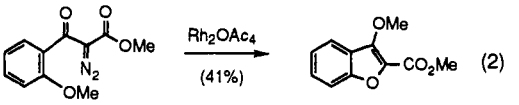
[a] (a) SO_2Cl_2, 68% (ref 7); (b) Ac_2O, pyridine, 93%; (c) $AlCl_3$, chlorobenzene, reflux, 30 min, 94%; (d) (*R*)-pent-3-en-2-ol, diisopropylazodicarboxylate, tributylphosphine, 73%; (e) 2 equiv of LHMDS, $NCCO_2Me$, THF, -78 °C, 78%; (f) MsN_3, Et_3N, 95%; (g) $Rh_2(piv)_4$, benzene, 1 h, reflux, 62%; (h) (1) O_3, CH_2Cl_2/MeOH, DMS; (2) $Ph_3P{=}C(CH_3)CO_2$-*t*-Bu, THF, room temperature, 70%; (3) TFA, 100%; (4) diphenyl phosphorazidate, Et_3N; reflux, toluene, then HCl/H_2O, reflux, 2 h, 80%; (i) NaOMe, MeOH, room temperature, 49%; (j) CH_2N_2, THF, room temperature, 95% (3:2 mix).

ee (prepared by resolution).[9] Asymmetry is thereby introduced and used in the elaboration of the spirocyclic stereocenter. The *S* ether **3** ($[\alpha]_D$ +22.8°) is methoxycarbonylated using Mander's reagent,[10] and the resulting β-keto ester is subjected to diazo transfer[11] to provide the key diazo ketone intermediate ($[\alpha]_D$ -6.9°). Decomposition of **4** is conducted with 5 mol % of rhodium pivalate catalyst in refluxing benzene for 1 h, providing the sigmatropic rearrangement product **5** ($[\alpha]_D$ -59.9°) in 62% yield after purification. No other stereoisomers or regioisomers can be detected in the crude reaction mixture. The stereochemistry of this process can be understood in terms of a transition-state model **8** that resembles an oxabicyclo[3.3.0]octane ring system with the key, stereochemistry-defining methyl group located on the convex face. The synthesis is completed by conversion of **5** to methyl ketone **6** ($[\alpha]_D$ +120.6°), which in racemic form has earlier served as an intermediate in griseofulvin synthesis.[5d] Its Dieckmann cyclization is conducted under superior conditions to provide griseofulvic acid (**7**) and thence griseofulvin. The material obtained (5% from **1**) is identical by NMR, IR, TLC (4:1 ethyl acetate/hexane), and optical rotation to the natural product.



8

Oxonium ylide generation from substrate **4** raises an important issue of reaction selectivity because there are two *o*-alkoxy groups. Ylide formation might occur at either, with the product ratio being determined by their relative rates of formation and available reaction pathways. To discern the possible fate of an *O*-methyl ylide, we prepared a diazo ketone from *o*-anisic acid. The product of its rhodium-catalyzed decomposition, isolated in 41% yield, is a benzofuran derived from 1,4-migration (eq 2). In the case of the griseofulvin synthesis, no products derived from the *O*-methyl ylide could be found. This result suggests that a Curtin-Hammett situation obtains. The symmetry-allowed [2,3]-sigmatropic rearrangement pathway having the lowest activation barrier is exclusively observed. Any *O*-methyl ylide formed from **4** must reverse.



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Supplementary Material Available: Experimental procedures and spectral data for **1-7** (4 pages). Ordering information is given on any current masthead page.

Competitive Energy and Electron-Transfer Quenching of Excited Ruthenium Polypyridyls by Ferrocene Derivatives

Eric J. Lee and Mark S. Wrighton*

Department of Chemistry
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139

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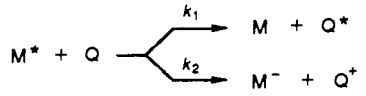
We report results showing that quenching of $Ru(bpy)_3^{2+}$ (bpy = 2,2′-bipyridine) and $Ru(bpz)_3^{2+}$ (bpz = 2,2′-bipyrazine) by ferrocene and several methyl derivatives occurs competitively by both energy and electron transfer, eq 1. The relative fraction of electron transfer to total quenching can be tuned by varying the



(1)

driving force for electron transfer as anticipated by Marcus theory.[1] The series of quenchers used is ferrocene (Fc), 1,1′-dimethylferrocene (Me_2Fc), 1,2,3,4,5-pentamethylferrocene (Me_5Fc), 1,1′,2,2′,3,3′,4,4′-octamethylferrocene (Me_8Fc), and decamethylferrocene ($Me_{10}Fc$). This series is useful in our study because the $E_{1/2}$ values vary from 0.38 to -0.11 V vs SCE in CH_3CN/0.1 M $[n\text{-}Bu_4N]PF_6$, whereas the lowest triplet excited states of ferrocenes are ligand field states of nearly the same energy, 1.8 eV.[2] The oxidizing power of excited $Ru(bpy)_3^{2+}$ and $Ru(bpz)_3^{2+}$ is 0.80 V vs SCE and 1.36 V vs SCE, respectively,[3] whereas the lowest excited-state energies, 2.15 eV, are essentially the same.[4] Therefore variation of either ferrocene quencher or

* Address correspondence to this author.

(1) (a) Marcus, R. A.; Sutin N. *Biochim. Biophys. Acta* **1985**, *811*, 265-322. (b) Mikkelsen, K. V.; Ratner, M. A. *Chem. Rev.* **1987**, *87*, 113-153. (c) Isied, S. S. *Prog. Inorg. Chem.* **1984**, *32*, 443-517.

(2) (a) Sohn, Y. S.; Hendrickson, D. N.; Gray, H. B. *J. Am. Chem. Soc.* **1971**, *93*, 3603-3612. (b) Kikuchi, M.; Kikuchi, K.; Kokubun, H. *Bull. Chem. Soc. Jpn.* **1974**, *47*, 1331-1333. (c) Herkstroeter, W. G. *J. Am. Chem. Soc.* **1975**, *97*, 4161-4167. (d) Farmilo, A.; Wilkinson, F. *Chem. Phys. Lett.* **1975**, *34*, 575-580. (e) Bhattacharyya, K.; Ramaiah, D.; Das, P. K.; George, M. V. *J. Phys. Chem.* **1986**, *90*, 5984-5989.

(3) (a) Crutchley, R. J.; Lever, A. B. P. *Inorg. Chem.* **1982**, *21*, 2276-2282. (b) Rillema, D. P.; Allen, G.; Meyer, T. J.; Conrad, D. *Inorg. Chem.* **1983**, *22*, 1617-1622. (c) Kawanishi, Y.; Kitamura, N.; Tazuke, S. *Inorg. Chem.* **1989**, *28*, 2968-2975.

(8) Hughes, D. L.; Reamer, R. A.; Bergan, J. J.; Grabowski, E. J. J. *J. Am. Chem. Soc.* **1988**, *110*, 6489.

(9) Hill, R. K.; Soman, R.; Sawada, S. *J. Org. Chem.* **1972**, *37*, 3737.

(10) Mander, L. N.; Sethi, P. S. *Tetrahedron Lett.* **1983**, *24*, 5425.

(11) Taber, D. F.; Ruckle, R. E.; Hennessy, M. J. *J. Org. Chem.* **1986**, *51*, 4077.

0002-7863/91/1513-8562$02.50/0