overall yield from **19**).[25] No $\Delta^{2,3}$ isomer was detected (Scheme IV). Thus, we have demonstrated that macrolactonization of the genuine seco acid **3a** can be essentially performed without isomerization.[26] Finally, the mixture of **21** and its epimer was treated with HF-pyridine to yield milbemycin α_1 (**1**: $R_f = 0.50$ on silica gel TLC, hexane:AcOEt = 1:1) in 65% yield and its chromatographically separable C2 epimer (13%; $R_f = 0.42$). The melting point (192-194 °C), $[\alpha]_D$ (+106°, acetone), and 600-MHz ^{1}H NMR, IR, and mass spectra of the synthetic **1** were identical with those of authentic milbemycin α_1 (mp 193-195 °C; $[\alpha]_D$ +107°).[5b]

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Supplementary Material Available: Spectroscopic data (^{1}H NMR, IR, MS and/or HRMS) and optical rotations for **8-19**, **3a**, **21**, and **1** as well as experimental procedures for transformations **8** → **9** → **10** and **19** → **3a** → **21** (11 pages). Ordering information is given on any current masthead page.

(25) Partial epimerization of C2 is likely to occur in the lactonization step, because the product **21** is stable under the reaction conditions.

(26) Intermediary formation of the β-lactone (Scheme I) has not yet been proved.

Enzyme-Modified Carbon-Fiber Microelectrodes with Millisecond Response Times

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Many strategies have been developed for the immobilization of enzymes on electrode surfaces to improve the selectivity of electrochemical measurements.[1] However, very few enzyme-modified electrodes have been constructed that afford both the small probe size (i.e., <10-μm diameter) and rapid response times necessary to meet the analytical requirements for monitoring dynamic neurochemical events (e.g., neurotransmitter release, which occurs on the millisecond time scale). Platinized microelectrodes utilizing surface-adsorbed enzymes have been reported to have response times on the order of a few seconds.[2] The stability of the enzyme-modified electrode has been improved by covalent attachment of the enzyme either to the electrode surface[3] or to redox polymers adsorbed to the electrode surface.[4] Here we present a strategy for linking an enzyme covalently to the surface of a carbon-fiber microelectrode (8-μm diameter) via a hydrophilic tether using biotin/avidin/biotin as a coupling technique. The biotin/avidin system is useful in that extremely strong binding ($K_D = 10^{-15}$ M) of four molecules of biotin to one of avidin allows the assembly of a molecular "sandwich", which serves to bind two components together.[5] Since the enzyme is linked through a tether that is covalently attached to the surface, this strategy allows much more control over the extent of coverage and the localization of the enzyme on the surface than does adsorption, without creating a diffusional barrier. This strategy should allow utilization of the vast number of available enzymes that can be linked to redox processes for the fabrication of single-component-selective microelectrodes with millisecond response times.

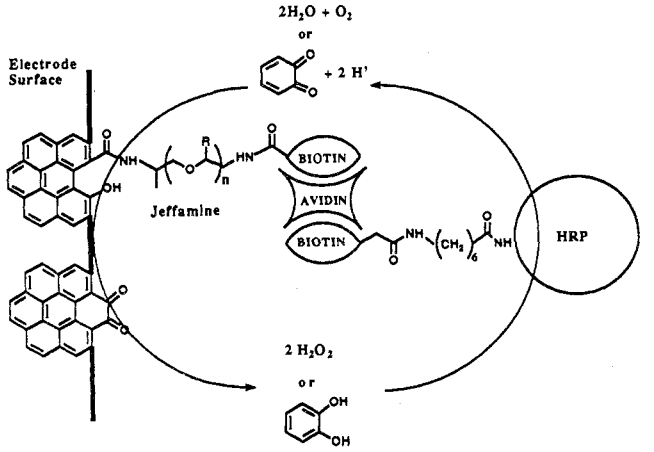

Figure 1. Schematic representation of the derivatization of the carbon surface with a hydrophilic tether (Jeffamine ED-600, whose extended length is 30-50 Å),[13] which is biotinylated and coupled to biotinylated horseradish peroxidase (HRP) via binding of the two biotins with one molecule of avidin.

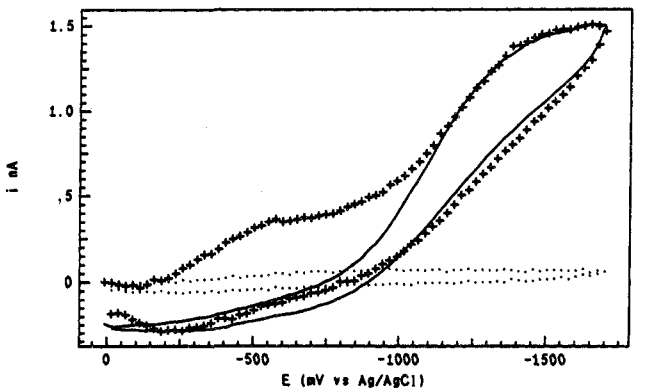

Figure 2. Background-subtracted cyclic voltammogram (25 V s^{-1}) of 10 averaged scans for 250 μM O_2 [saturated phosphate buffer (pH 7.4) at 25 °C; current scale is twice that shown] (—); enzyme-generated O_2 from 2.5 mM H_2O_2 (+++); and response observed after deactivation of surface-bound HRP by boiling modified electrode in water for 10 min [···].

Carbon-fiber microelectrodes (Thornel P-55S) were prepared as described previously by Kelly and Wightman.[6] Carboxyl groups on the carbon surface were activated by reacting with 4 mg/mL of 1-ethyl-3-[(dimethylamino)propyl]carbodiimide in phosphate buffer (0.15 M NaCl, 0.10 M Na_2HPO_4, pH 7.4) for 12 h. The electrodes were then dipped into a 0.4 mM solution of Jeffamine ED-600 (a poly(oxyalkylene)diamine, Texaco) in aqueous buffer for 30 min. This attaches the hydrophilic spacer arm to the electrode surface via an amide bond. Following a buffer wash, the electrodes were placed in a 1 mg/mL solution of Sulfo-NHS-Biotin (Pierce Chemical) in phosphate buffer (pH 7.4) for 5 h to attach a biotin to the free terminal amines on the surface-bound Jeffamines. After rinsing with buffer, the electrodes

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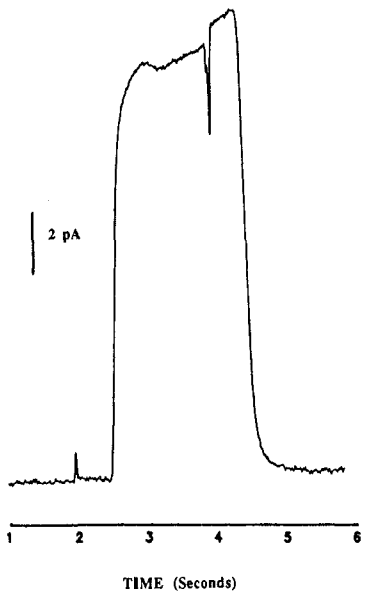

Figure 3. Time course of quinone generation at a HRP-modified electrode. Cathodic current monitored at -0.300 V (dc, 200-ms time constant) for the injection of 10 mM catechol (FIA flow rate 1 mL min^{-1}). The spikes represent the beginning and end of sample injection (the delay to response corresponds to the dead time of the injection system).

were dipped into a premixed solution containing 3 μM ExtrAvidin (Sigma) and 0.3 μM biotinylated horseradish peroxidase (HRP, Pierce Chemical) for 12 h at 4 °C to complete the derivatization (Figure 1).

Surface derivatization can create density of one tether molecule/220 Å^2,[7] almost 10 times greater than necessary for complete surface coverage with avidin.[8] The electrochemical properties of the HRP-modified electrode were examined by flow injection analysis.[9] Cyclic voltammetry (25 V s^{-1}) was used to provide selective detection of oxygen generated from the autocatalysis of hydrogen peroxide. The enzymatically generated product (Figure 2) was voltammetrically identical with molecular oxygen.[10] The response was linear for H_2O_2 between 5×10^{-3} and 4×10^{-5} M H_2O_2 ($r^2 = 0.989$, $n = 5$), with a detection limit of 10^{-5} M H_2O_2. The selectivity of the measurement was demonstrated by inactivating the surface-bound HRP by boiling the electrode in water for 10 min, After this treatment, the electrode response to hydrogen peroxide was abolished without any loss in sensitivity to molecular oxygen (Figure 2).

The response time of the electrode assembly was evaluated by using catechol as the substrate for HRP catalysis in a flow injection experiment with dc amperometric detection of the enzyme-generated quinone, The $E_{1/2}$ of the catechol/quinone couple was determined by fast-scan cyclic voltammetry (250 V s^{-1}) to be 0.187 V vs Ag/AgCl. Therefore, the electrode was poised at -0.300 V vs Ag/AgCl (to ensure complete electrolysis of the quinone generated by the surface-bound HRP) and the cathodic current was monitored continuously versus time, Injection of a 2-s pulse of 10 mM catechol showed a response time less than 300 ms (244 ± 43 ms, $n = 5$) for the HRP-modified electrode (Figure 3), Thus, attachment of an enzyme via a hydrophilic tether through a biotin/avidin coupler produces an electrode that has the size, speed, and sensitivity required for dynamic measurements of in vivo biochemical processes, and the surface coverage of the enzyme can be closely controlled by adjusting the conditions of the surface derivatization reaction.

The other virtue of this immobilization scheme is that the peroxidase can be easily replaced by other enzymes that liberate other redox species. Ultimately, this should lead to an array of well-characterized chemical sensors with rapid response times, each specific for the measurement of a single component in a complex chemical matrix. Of course, the in vitro measurements described here are not as complex as would be expected in vivo. Placement of an electrode into a biological tissue often results in severe degradation of analytical performance due to surface fouling. Nevertheless, a great deal of meaningful information has already been obtained in short-duration experiments (i.e., 4-12 h) with unmodified[11] and enzyme-modified[12] microelectrodes placed in vivo in the mammalian brain.

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(7) The extent of surface coverage was assessed by derivatizing the surface with an electroactive amine, 3,4-dihydroxyphenethylamine (dopamine), in place of the Jeffamine ED-600. Chronocoulometry (-0.4 V to +0.4 V, τ = 100 ms) showed a 50-pC increase in the charge observed at the carbon surface due to the oxidation of surface-bound dopamine. This corresponds to one dopamine molecule/220 Å^2 (based on a geometrical surface area of 350 μm^2), similar to that found by Chi-Sing Tse and Kuwana (*Anal. Chem.* **1978**, *50*, 1315-1318) for macroscopic glassy carbon electrodes. Electrochemical measurements were performed on an EI-400 potentiostat (Ensman Instruments, Bloomington, IN) where all voltammetric waveforms were generated and current acquired via a PC-AT microcomputer (Zenith Data Systems) interfaced via an A/D//D/A interface (Labmaster DMA, Scientific Solutions, Solon, OH).

(8) The cross-sectional area of avidin can be calculated from crystallographic data to be on the order of 2000 Å^2 (Weber, P, C.; Ohlendorf, D. H.; Wendoloski, J. J.; Salemme, F. R. *Science* **1989**, *243*, 85-88).

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(10) All flow injection components were stainless steel to prevent atmospheric oxygen from entering into the system. All solutions were rigorously degassed with nitrogen before use.

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Insertion Chemistry of $Cp_2Zr(\eta^2\text{-}C,N\text{-}CH_2\{6\text{-Me-pyrid-2-yl}\})^+$: Facile Zirconium-Mediated Functionalization of Methyl C-H Bonds of 2,6-Lutidine

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The rich insertion chemistry of three-membered metallacycles such as Cp_2M(benzyne), Cp_2M(olefin), and Cp_2M(pyridyl)$^+$ (M = group 4 metals) provides a general approach to metal-mediated C-C bond formation.[1-3] In contrast, the insertion chemistry of

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