Communications to the Editor

Simple Strategy for the Synthesis of the Avermectin–Milbemycin Family. Total Synthesis of Milbemycin α_1

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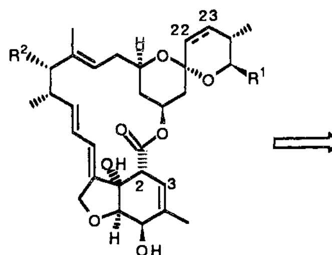
Received October 9, 1990

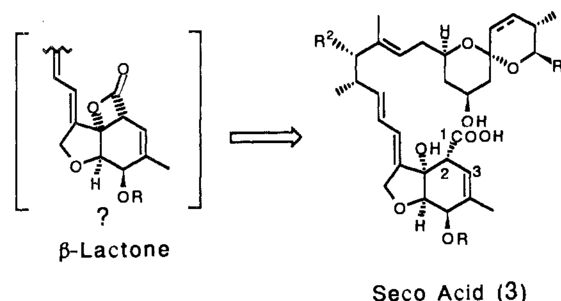
The avermectins[1] and the milbemycins[2] have attracted considerable interest with regard to the synthesis[3] because of their unique structures and potent antiparasitic activities.[4] Construction of the hexahydrobenzofuran moiety with both the correct stereochemistry (particularly at C2) and the C3–C4 double bond is the most important and challenging aspect in their total synthesis, because this structure, prone to undergo C2 epimerization, double-bond migration, and dehydration,[5] is essential for their biological activity.[3] Successful total syntheses reported thus far have adopted secure strategies introducing the crucial 3,4-double bond at the final stages after constructing the whole macrolactone structures.[3,6,7] These approaches utilizing the deconjugation–epimerization[5c,6] or the selenenylation–elimination process,[7] however, are still less than satisfactory in terms of stereo- and regiocontrol. Thus, we have attempted a simpler but apparently more difficult approach toward this family through the macrolactonization of a genuine seco acid such as **3** on the working hypothesis that it could be executed without epimerization/conjugation by utilizing a β-lactone intermediate (Scheme I). We describe herein the straightforward synthesis of milbemycin α_1 (**1**).

It was necessary to increase the oxidation state of C1 at least to the aldehyde level before coupling the hexahydrobenzofuran subunit[8] with the spiroketal subunit,[9] since it had turned out to be difficult to obtain the seco aldehyde or the seco acid from the corresponding homoallylic alcohol **4**.[10] Therefore, several homoallylic alcohols were synthesized and tested for oxidation. Alcohols **5**[8] and **8**[8] were successfully oxidized to **6** and **9**, respectively (≥70% yield), through DMSO oxidation[11] under degassed conditions.[12] The dioxane derivative **6** was stable at room temperature

Scheme I



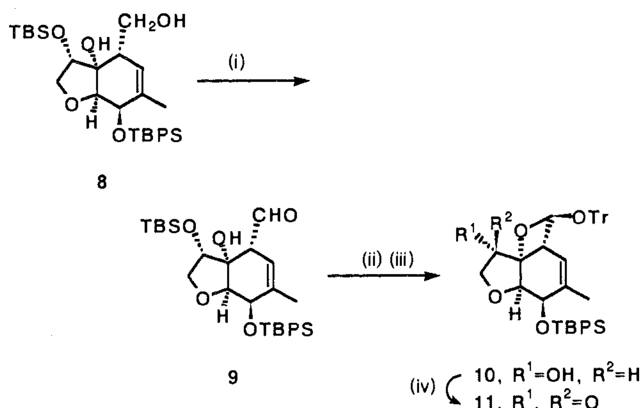
Milbemycin α1 (1)
(R^1=Me; R^2=H; 22,23-dihydro)
Milbemycin D (2)
(R^1=i-Pr; R^2=H; 22,23-dihydro)
Avermectins
(R^1=alkyl; R^2=glycoside; $\Delta^{22,23}$)



β-Lactone

Seco Acid (3)

Scheme II[a]



[a] Reagents and conditions: (i) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −78 °C; (ii) TrOTf (2.5 equiv), 2,6-lutidine (5 equiv), CH_2Cl_2, 0 °C; (iii) n-Bu_4NF (2 equiv), 0 °C, 4 h; (iv) $(CF_3CO)_2O$, DMSO, Et_3N, CH_2Cl_2, −65 °C.

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(10) All attempts to oxidize **4** failed by any means (PCC, PDC, CrO_3·2py, Swern oxidation,[11] and so on); Fraser-Reid observed that PCC oxidation of related homoallylic alcohols led to the allylic cleavage to give enone.[7b] Susceptibility of the diene system to decomposition under the oxidation conditions (detected by NMR) might be one of the major causes of the failure (cf.: Selnick, H. G.; Danishefsky, S. J. *Tetrahedron Lett.* **1987**, *28*, 4955).
(11) Swern oxidation (Mancuso, A. J.; Huang, S.-L.; Swern, D. *J. Org. Chem.* **1978**, *43*, 2480) was the best.

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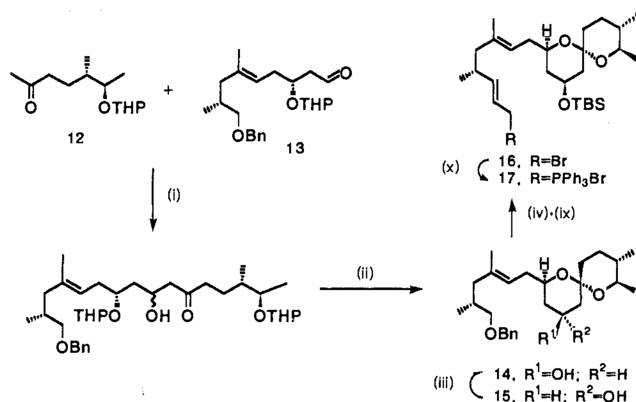
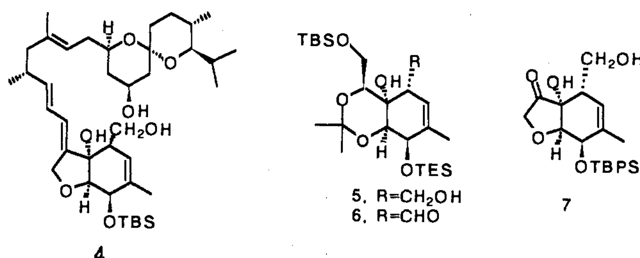
Scheme III[a]



[a] Reagents and conditions: (i) **12**, LDA (1.2 equiv), THF, -78 °C, then **13** (1 equiv), -78 °C, 15 min; (ii) p-TsOH·H_2O (cat.), MeOH; (iii) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, -60 °C → 0 °C, and then t-$BuNH_2$·BH_3 (15 equiv); (iv) TBSCl, imidazole, DMF; (v) Na, liquid NH_3, -78 °C; (vi) $(COCl)_2$, DMSO, Et_3N, -60 °C; (vii) Ph_3PCHCO_2Me (2 equiv), C_6H_6, 12 h; (viii) DIBAL (2.5 equiv), Et_2O, -78 °C; (ix) CBr_4 (2 equiv), PPh_3 (2.5 equiv), CH_2Cl_2, -20 °C, 5 min; (x) PPh_3 (2 equiv), CH_3CN, 50 °C, 2 h.

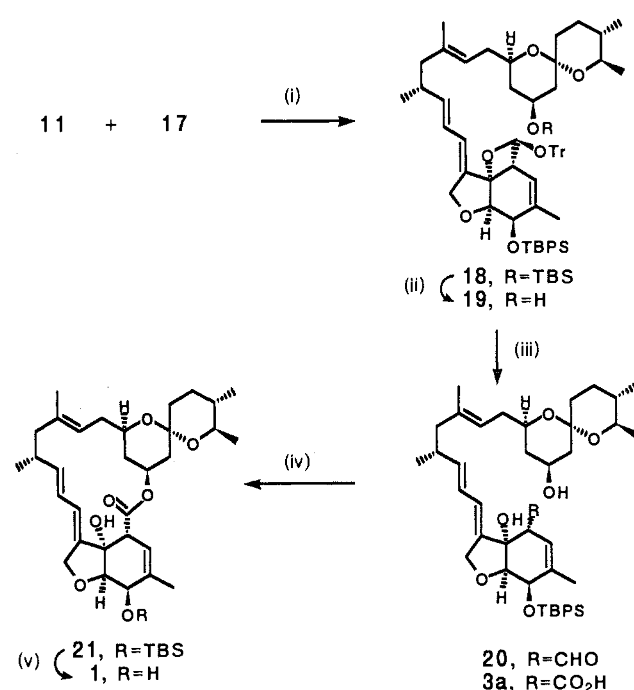
and purified on silica gel chromatography. The aldehydes possessing a tetrahydrofuran ring, however, became less stable[13] so that **9** could not tolerate chromatographic purification. In particular, the aldehydes with a C8 sp^2 center on the hexahydrobenzofuran ring were too labile to be manipulated or to isolated. Thus, oxidation of **7**[8] was unsuccessful and gave only a complex mixture. This result coincides with the failure of the oxidation of **4**.[10] Further oxidations of the aldehydes **6** and **9** to the corresponding acids have been accomplished in good yield through $NaClO_2$ oxidation[14] without isomerization.



New methodology to protect the labile β-hydroxy aldehyde functionality has been invented. Treatment of crude **9** with sterically demanding trityl triflate[15] in the presence of 2,6-lutidine yielded the trityl oxetane acetal **10**[16] (74% overall yield from **8**) after desilylation of the *tert*-butyldimethylsilyl (TBS) ether. Then, DMSO–$(CF_3CO)_2O$ oxidation[17] of **10** afforded a key intermediate **11** (82%), adequate for coupling with the spiroketal subunit **17** (Scheme II).

Synthesis of **17** was carried out as shown in Scheme III. Aldol coupling of ketone **12**[18] with aldehyde **13**[19] followed by acid hydrolysis produced the desired β-alcohol **14** (30–36% overall yield; R_f = 0.25 on silica gel TLC, hexane:AcOEt = 4:1) and readily separable epimer **15** (13–16%; R_f = 0.60), which was converted to **14** (75% yield) through Swern oxidation and subsequent reduction with t-$BuNH_2$·BH_3 in one pot.[21] Bromide **16** was synthesized from **14** by employing standard methodology (six steps, 76% overall yield) and transformed quantitatively to triphenylphosphonium bromide **17** in acetonitrile.

Scheme IV[a]



[a] Reagents and conditions: (i) **17** (3 equiv), n-BuLi (3.1 equiv), THF, -60 °C, then **11**, -60 °C → -20 °C, 2 h; (ii) n-Bu_4NF (6 equiv), 1 h; (iii) HCO_2H, t-BuOH, 2-methyl-2-butene (1:10:10), 1.5 h; $NaClO_2$ (20 equiv), NaH_2PO_4, H_2O, 1 h; (iv) Et_3N (5 equiv), DMAP (2 equiv), 2,4,6-$Cl_3C_6H_2COCl$ (1.2 equiv), toluene, 1 h; (v) HF, pyridine (1:2), 36 h.

Since the tactics and the materials were in hand, we attempted our original synthetic plan. Ketone **11** was reacted with an ylide prepared from **17** to afford the conjugated diene **18** as a single product in 39% yield. Its TBS group was removed to give the alcohol **19** (73%). Deprotection of the oxetane acetal **19** with formic acid[22,23] in t-BuOH and 2-methyl-2-butene (1:1) and subsequent oxidation through direct addition of this mixture into the aqueous solution of $NaClO_2$ and NaH_2PO_4[14,23] without isolation of labile aldehyde **20** gave the homogeneous seco acid **3a** in 40% overall yield after chromatography on Florisil, while in the sequence of total synthesis **3a** was not purified. The last critical lactonization of the crude seco acid **3a** was achieved by the modified Yamaguchi method[24] utilizing 2,4,6-trichlorobenzoyl chloride, triethylamine, and 4-(dimethylamino)pyridine (DMAP) at room temperature (1 h) to afford the TBS ether (**21**) of milbemycin α_1 together with its C2 epimer in a 5:1 ratio (22%

(12) Otherwise **6** and **9** reacted further with oxygen to form the aldehydes with $\Delta^{2,3}$-allylic hydroperoxides.

(13) Stability of those cyclohexene carbonyl derivatives appears to be highly dependent on the presence and the structure of a condensed ring system. Monocyclic systems seemed to be more stable; see: Parmee, E. R.; Steel, P. G.; Thomas, E. J. *J. Chem. Soc., Chem. Commun.* **1989**, 1250.

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(16) Corresponding triisopropylsilyl oxetane acetal was also prepared by using triisopropylsilyl triflate-lutidine; any evidence for hemiacetal formation in **9** was not observed.

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(18) Synthesized by alkylation of the lithiated *N,N*-dimethylhydrazone of acetone with (2*R*,3*R*)-1-iodo-2-methyl-3-(tetrahydropyranyloxy)butane, which was prepared through chelation-controlled cuprate attack (Schneider, J. A.; Still, W. C. *Tetrahedron Lett.* **1980**, *21*, 1035). For a related synthesis, see: Baker, R.; O'Mahony, M. J.; Swain, C. J. *Tetrahedron Lett.* **1986**, *27*, 3059.

(19) Synthesized from (2*R*)-1-(benzyloxy)-2-methyl-4-pentyne and (2*S*)-1,2-epoxy-4-[(4-methoxyphenyl)methoxy]butane, prepared from methyl (*S*)-malate.[20] For a related synthesis, see: Culshaw, D.; Grice, P.; Ley, S. V.; Strange, G. A. *Tetrahedron Lett.* **1985**, *26*, 5837.

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(21) The ratio **18**:**19** was 82:18 in 93% yield.

(22) Bessodes, M.; Komiotis, D.; Antonakis, K. *Tetrahedron Lett.* **1986**, *27*, 579.

(23) Since the standard deprotection procedure according to Bessodes[22] (formic acid in ether, then removal of the volatiles in vacuo) resulted in deterioration of the aldehyde **20**, the conditions for the deprotection and subsequent oxidation are modified.

(24) Hikota, M.; Tone, H.; Horita, K.; Yonemitsu, O. *J. Org. Chem.* **1990**, *55*, 7.

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]

Acknowledgment. We are grateful to Mr. Shoichi Takeishi for preliminary studies on the route to **14**, to Dr. Hiroshi Mishima (Sankyo Co.) for a generous gift of milbemycin α_1 and D, to Prof. Osamu Yonemitsu (Hokkaido University) for detailed experimental procedures of macrolactonization, and to Dr. Masako Ueno and Mr. Kazuo Sasaki and to Mr. Toshio Sato (Instrumental Analysis Center for Chemistry of Tohoku University) for NMR and FABMS measurements, respectively.

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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Received October 29, 1990

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.

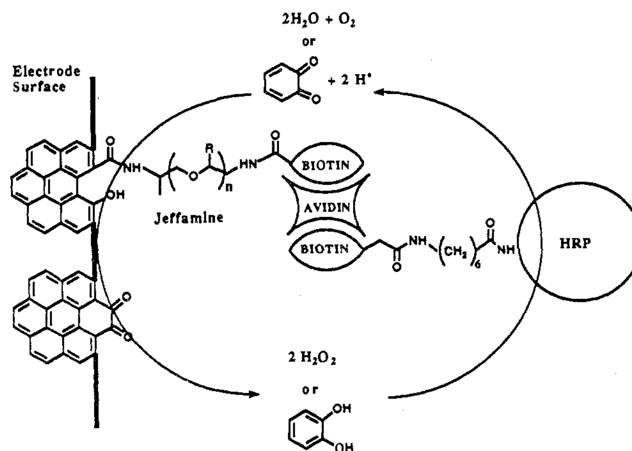

Figure 1. Schematic representation of the derivatization of the carbon surface with a hydrophilic tether (Jeffamine ED-600, whose extended length is 30-50 Å),[13] which is biotinylated and coupled to biotinylated horseradish peroxidase (HRP) via binding of the two biotins with one molecule of avidin.

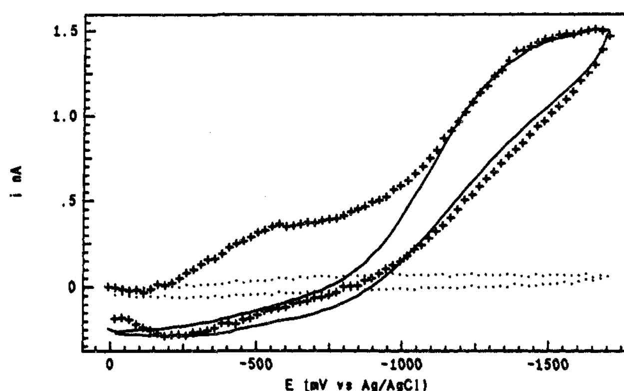

Figure 2. Background-subtracted cyclic voltammogram (25 V s^{-1}) of 10 averaged scans for 250 μM O_2 [saturated phosphate buffer (pH 7.4) at 25 °C; current scale is twice that shown] (—); enzyme-generated O_2 from 2.5 mM H_2O_2 (+++); and response observed after deactivation of surface-bound HRP by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