Synthesis of the Antifungal Agent Norneoenactin A

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Received June 14, 1993•

The first total and unequivocal syntheses of (*R*)- and (*S*)-norneoenactin A (1), an analog of the antifungal antibiotic neoenactin A, are described. The key step of each synthesis was the Michael addition of a serinehydroxamate (5 or 8) to the appropriate vinyl ketone 4. Biological activity of the two enantiomers of norneoenactin A ((*S*)-1 and (*R*)-1) against *Escherichia coli* and several fungi has been investigated.

Introduction

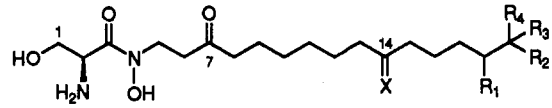
Enactins (ENs) and neoenactins (NEs), produced by *Streptomyces roseoviridis* and *Streptoverticillium olivoreticuli*, respectively, are L-serinehydroxamic acid-based antimycotic antibiotics potentiating the action of polyene antifungal antibiotics and antitumor agents such as bleomycin and vincristine.[1] Therefore, ENs and NEs are given the group name hydroxamic acid antimycotic antibiotics (HAAA).[1b] Although the biological properties and chemical structures (Figure 1) of ENs and NEs are very similar, NEs are more hydrophobic and more active against fungi.

Thus far, several congeners of NEs have been isolated, and all of them are almost equally active against many organisms.[2] Our synthetic efforts were therefore focused on the main component of NEs, neoenactin A (NE A), and analogs. Herein, we describe the first total synthesis of the two enantiomers of norneoenactin A (1), an analog of NE A which contains one less methylene group between carbonyls 7 and 14 (Scheme I).

Results and Discussion

In our approach toward the synthesis of norneoenactin A, we set out to devise a convergent flexible synthesis which should provide NE A and a number of analogs. As the retrosynthetic analysis of the planned synthesis shows (Scheme I), our goal should be accomplished rather easily. Disconnection of 1 at the hydroxamic nitrogen provides protected serinehydroxamic acid and a vinyl ketone. The vinyl ketone could be obtained from the corresponding acid and serinehydroxamic acid from a protected form of the amino acid serine.

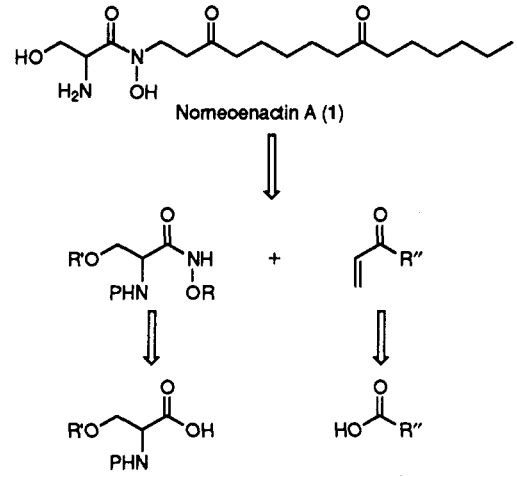
Synthesis of the vinyl ketone 4 started with 2-heptanoylcyclohexanone (2, Scheme II),[3] which was obtained in 78% yield by refluxing a solution of 1-morpholinocyclohexene[4] and heptanoyl chloride in the presence of triethylamine in benzene followed by acid hydrolysis of the resulting salt. Treatment of 2 with refluxing aqueous potassium hydroxide provided 7-oxotridecanoic acid (3)[5] in 80% yield. Conversion of acid 3 to the Michael acceptor



	X	R_1	R_2	R_3	R_4
EN Ia	O	H	Me	Me	OH
EN Ib_1, Ib_2	O	Me	Me	H	OH
EN Va	OH, H	Me	H	H	H
NE A	O	H	Me	H	H
NE M_1	O	Me	H	H	H
NE M_2	OH, H	H	Me	H	H
NE B_1	O	Me	Me	H	H
NE B_2	O	H	Me	Me	H
NE NL_1	O	H	H	H	H
NE NL_2	O	H	Et	H	H

Figure 1.

Scheme I



vinyl ketone 4 was then desired. Soderquist and Leong[6] reported the palladium-catalyzed cross-coupling of vinylstannanes with acid chlorides to give vinyl ketones in good to excellent yields. Accordingly, acid 3 was converted to the acid chloride which was then treated with tri-*n*-butylvinylstannane in the presence of a catalytic amount of *trans*-benzyl(chloro)bis(triphenylphosphine)-palladium(II) in refluxing benzene to give the vinyl ketone 4 in moderate yields.

• Abstract published in *Advance ACS Abstracts*, September 1, 1993.

(1) Leading references: (a) Yamamoto, K.; Shiinoki, Y.; Furukawa, J.; Nakamura, S. *Chem. Pharm. Bull.* **1991**, *39*, 1436 and references cited therein. (b) Okada, H.; Yamamoto, K.; Tsutano, S.; Inouye, Y.; Nakamura, S.; Furukawa, J. *J. Antibiot.* **1989**, *42*, 276 and references cited therein.

(2) Roy, S. K.; Inouye, Y.; Nakamura, S. *J. Antibiot.* **1987**, *40*, 266.

(3) Pratap, G.; Shantha, K. L.; Rao, V. S. B. *Fett Wiss. Technol.* **1989**, *91*, 68.

(4) Stork, G.; Brizzolara, A.; Landesman, H.; Szmuszkovicz, J.; Terrell, R. *J. Am. Chem. Soc.* **1963**, *85*, 207.

(5) (a) Pratap, G.; Shantha, K. L.; Rao, V. S. B.; Krishnamurti, N. *J. Appl. Polym. Sci.* **1990**, *41*, 945 (physical data for this compound were not reported as of this date). (b) Kawamura, K.; Gagosian, R. B. *J. Chromatogr.* **1988**, *438*, 299. (c) Hamrick, P. J., Jr.; Hauser, C. F.; Hauser, C. R. *J. Org. Chem.* **1959**, *24*, 583.

(6) Soderquist, J. A.; Leong, W. W.-H. *Tetrahedron Lett.* **1983**, *24*, 2361.

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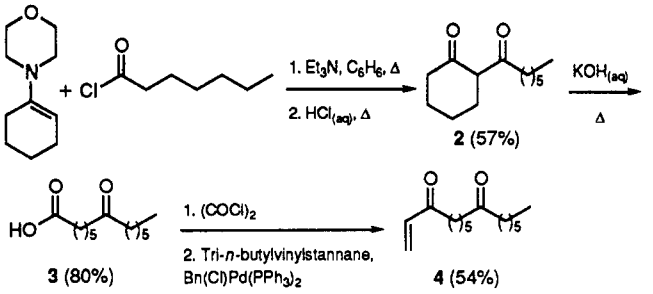
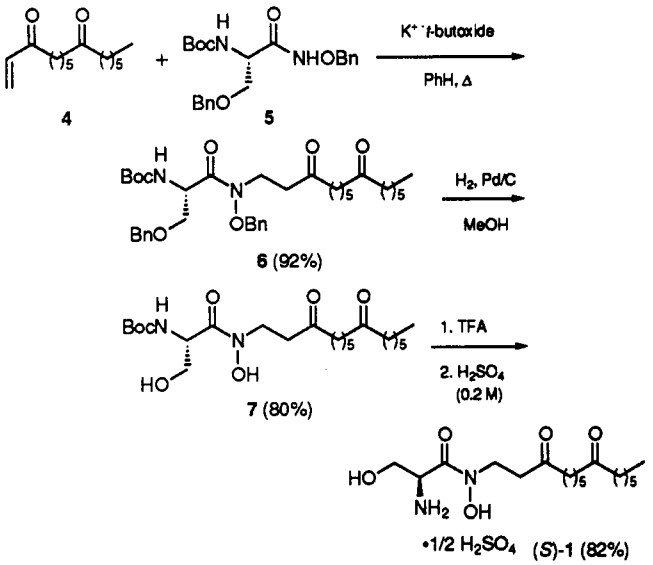
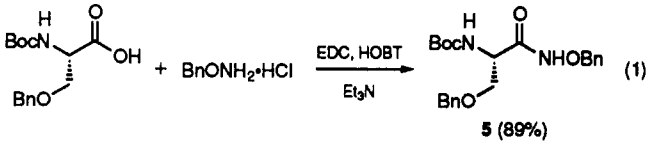
Scheme II



Scheme III



With the vinyl ketone **4** in hand, attention was turned to the synthesis of benzyl-*N*-(*tert*-butyloxycarbonyl)-*O*-benzyl-(*S*)-serinehydroxamate (**5**) required for the Michael reaction. Olsen *et al.*[7] reported the only synthesis of **5** in 82% yield by coupling of *O*-benzyl-*N*-(*tert*-butyloxycarbonyl)-(*S*)-serine and *O*-benzylhydroxylamine using 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide (EDC, eq 1).



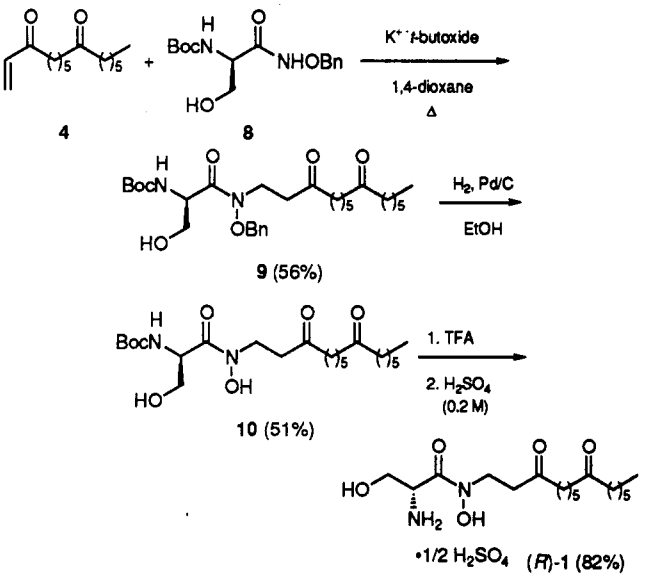
(1)

In our laboratories, the coupling reaction provided pure **5** in 89% yield with no need for chromatographic purification.

Since both **4** and **5** were readily available, the Michael reaction was then explored (Scheme III). Refluxing a mixture of **4** and **5** in benzene for 24–48 hours in the presence of a catalytic amount of potassium *tert*-butoxide produced the desired Michael adduct **6** in moderate yields.

With an abundant supply of protected norneoenactin A (**6**), reductive removal of the benzyl groups was then attempted. Treatment of **6** with 10% palladium on carbon in methanol under a hydrogen atmosphere resulted in the rapid deprotection of the hydroxamic acid (starting material was consumed within 1 h). However, reduction of the benzyl ether functionality, without the concomitant reduction of the N–O bond, required fine tuning of the reduction conditions. When the reduction was carried out using 20–25 mol % of 10% Pd/C at 20 or 30 mM concentrations for 22–31 h, a mixture of two inseparable products was obtained. One was believed to be the desired debenzylated product and the other the deoxygenated product resulting from overreduction of the N–O bond. Catalytic reduction with 10% Pd/C (30 mol %) in dioxane over 3 days provided a mixture of the desired debenzylated and monobenzylated products as well as the starting material. Finally, carrying out the reaction with 30 mol % of 10% Pd/C at 6–8.6 mM concentrations for 11–16 h provided the pure debenzylated product **7** in 80% yield after column chromatography.

Scheme IV



The final step of this synthesis involved removal of the Boc group. Since neoenactin congeners were isolated as their sulfate salts,[2] direct conversion of the protected norneoenactin A to its sulfate salt was unsuccessfully attempted by reaction of **7** with sulfuric acid at various temperatures, concentrations and times. Compound **7** was then treated with a 1:1 solution of trifluoroacetic acid/methylene chloride, and the resulting TFA salt was treated with dilute sulfuric acid solution. This procedure resulted in precipitation of the product (*S*)-**1** as a white solid which was ninhydrin and ferric chloride positive. The IR spectrum of the synthetic (*S*)-**1** was essentially identical to the one published for neoenactin A, the naturally occurring homolog.[8]

Thus, the synthesis of the nor-analog (S)-**1** of neoenactin A was accomplished. In our attempts to determine if both enantiomers were active antifungal agents, we decided to synthesize the enantiomer of norneoenactin A, (*R*)-norneoenactin A. However, due to the difficulty encountered with reduction of the benzyl ether functionality in **6** without the concomitant reduction of the N–O bond, we decided to carry out the Michael reaction of the vinyl ketone **4** with free hydroxy serinehydroxamate **8** instead of the fully protected enantiomer of serine derivative **5**.

Thus, benzyl-*N*-(*tert*-butyloxycarbonyl)-D-serinehydroxamate (**8**, Scheme IV)[9] was treated with excess vinyl ketone **4** in the presence of a catalytic amount of potassium *tert*-butoxide in refluxing 1,4-dioxane. The resulting

(7) Ramasamy, K.; Olsen, R. K.; Emery, T. *J. Org. Chem.* **1981**, *46*, 5438 (no experimental or physical data were reported for compound **5**).

(8) Nishio, M.; Yasuda, N.; Nakamura, S. *J. Antibiot.* **1983**, *36*, 1399.

(9) (a) Miller, M. J.; Mattingly, P. G.; Morrison, M. A.; Kerwin, J. F., Jr. *J. Am. Chem. Soc.* **1980**, *102*, 7026. (b) Kolasa, T.; Miller, M. J.; Okonya, J. F. Unpublished results.

yellow residue was purified by column chromatography (50% ethyl acetate/hexanes) to give the desired product **9** in 56% yield. The ^1H and ^{13}C NMR spectra were very similar to the corresponding spectra obtained for compound **6** which suggested that the isolated compound was the desired, and expected, *N*-alkylated product, not the one resulting from alkylation of the serine hydroxyl group.

Reduction of the benzyl group in **9** was then attempted. Treatment of **9** with 10% palladium on carbon in ethanol, under hydrogen atmosphere, for 105 min resulted in the deprotection of the hydroxamic acid to give *N*-Boc-D-norneoenactin A (**10**), in a moderate yield, as a white solid upon purification by column chromatography (3% methanol/ methylene chloride). The melting point and ^1H and ^{13}C NMR spectra of **10** were identical to the data obtained for *N*-Boc-(*S*)-norneoenactin A (**7**), to further prove that the product isolated from the Michael reaction was the desired *N*-alkylated one.

Deprotection of the amino group in **10** was carried out as described earlier. Treatment of **10** with a 1:1 solution of trifluoroacetic acid/methylene chloride provided a colorless oil upon evaporation of the volatiles. The resulting oil was then treated with a 1:1 solution of methylene chloride and 0.2 M sulfuric acid. The resulting white precipitate was collected to provide (*R*)-norneoenactin A [(*R*)-**1**] in 83% yield.

To determine the optical purity of the two isomers of **1**, the *o*-phthalaldehyde/*N*-acetylcysteine derivative of each enantiomer was prepared as reported in the literature.[10] Thus, the two synthetic isomers of norneoenactin A, which contain a primary amine, were transformed into fluorescent compounds and analyzed by a C18 reversed-phase analytical HPLC column equipped with a fluorescence detector. The results indicated that (*S*)-**1** was nearly enantiomerically pure (>96% ee), whereas (*R*)-**1** was 93% pure (86% ee).

The two isomers were tested against *E. coli* and a select number of fungi, including *Candida albicans*, *Aspergillus fumigatus*, and *Cryptococcus neoformans*. Although the two enantiomers of norneoenactin A were inactive against *E. coli*, (*S*)-norneoenactin A sulfate [(*S*)-**1**)] exhibited significant inhibitory activity (MIC < 1 μg/mL) against *Candida albicans* and *Aspergillus fumigatus*, comparable to neoenactin A itself. On the other hand, the (*R*) isomer failed to inhibit fungal growth at concentrations as high as 4.25 μg/mL. Full details of these results will be disclosed in due time.

Experimental Section

General Methods. Instruments and general chromatographic methods used have been described previously.[11]

1-Morpholinocyclohexene.[4] A solution of cyclohexanone (10.0 mL, 96.5 mmol) and morpholine (12.6 mL, 144.7 mmol) in benzene (10 mL) was refluxed for 8 h with azeotropic removal of water. The reaction mixture was allowed to cool to room temperature, washed with brine, dried ($MgSO_4$), and filtered, and the solvent was evaporated. The crude residue was distilled to afford 12.66 g (78%) of the desired product as a clear oil: bp 93 °C (1.5 mmHg), (lit.[4] bp 104–106 °C (12 mmHg)); ^1H NMR ($CDCl_3$) δ 4.70 (t, 1H), 3.70 (t, 4H), 2.70 (t, 4H), 2.00 (m, 4H), 1.60 (m, 4H).

2-Heptanoylcyclohexanone (2).[3] To a solution of 1-morpholinocyclohexene (3.17 g, 19 mmol) and triethylamine (2.63 mL, 19 mmol) in benzene (50 mL) was added heptanoyl chloride (2.9 mL, 19 mmol). The reaction mixture was refluxed for 12 h, after which time it was cooled to room temperature and HCl (10% solution) was added. The mixture was refluxed for 4 h, cooled to room temperature, washed with water, saturated sodium bicarbonate, and brine, dried ($MgSO_4$), and filtered and the solvent was evaporated. The crude residue was distilled to afford 2.27 g (57%) of **2** as a light yellow oil: bp 118–120 °C (0.60 mmHg), (lit.[3] bp 146 °C (6 mmHg)); IR (neat) 2950, 2860, 1640, and 1420 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.60 (m, 2H), 2.40 (m, 5H), 1.70 (m, 5H), 1.30 (m, 7H), 0.90 (t, 3H); ^{13}C NMR ($CDCl_3$) δ 201.2, 181.3, 106.3, 36.6, 31.4, 30.8, 28.8, 23.9, 23.5, 22.6, 22.2, 21.4, 13.7; HRMS calcd for $C_{13}H_{22}O_2$ 210.1620, found: 210.1623.

7-Oxotridecanoic Acid (3).[5] A solution of cyclohexanone **2** (3.91 g, 18.63 mmol) and excess 10% aqueous KOH was refluxed for 4 h. The reaction mixture was then cooled to room temperature, extracted with ethyl ether, and acidified to pH 1 with 6 N HCl. The acidic solution was then extracted with methylene chloride, after which the organic layer was dried ($MgSO_4$) and filtered and the solvent was evaporated. The crude residue was recrystallized from ethyl acetate/hexanes to afford 3.37 g (80%) of the desired acid **3** as a white crystalline solid: mp 60–61 °C; IR (KBr) 3140, 2960, 2940, 2860, 2660, 1700, and 1465 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.40 (m, 2H), 1.60 (m, 4H), 1.30 (m, 14H), 0.90 (t, 3H); ^{13}C NMR ($CDCl_3$) δ 211.4, 179.5, 42.6, 42.2, 33.7, 31.4, 28.7, 28.4, 24.2, 23.6, 23.2, 22.3, 13.8; HRMS calcd for $C_{13}H_{24}O_3$ 228.1725, found 228.1730. Anal. Calcd for $C_{13}H_{24}O_3$: C, 68.38; H, 10.59. Found: C, 68.17; H, 10.17.

3,9-Dioxopentadec-1-ene (4). To a solution of acid **3** (3.91 g, 17 mmol) in 50 mL of methylene chloride was added oxalyl chloride (4.0 mL, 5.82 g, 46 mmol) dropwise. The reaction mixture was then refluxed for 1.5 h and cooled to room temperature, and the volatiles were removed *in vacuo*. The resulting residue was redissolved in benzene (40 mL), and vinyltri-*n*-butylstannane (Fluka, 5.0 mL, 5.43 g, 17.1 mmol) and a catalytic amount of benzyl(chloro)bis(triphenylphosphine)palladium (II) were added. The resulting reaction mixture was refluxed for 3.5 h, after which time it was cooled to room temperature and the solvent was evaporated. The black residue was then washed through a pad of silica gel using hexanes (300 mL) followed by 10% ethyl acetate/ hexanes (500 mL). The second fraction contained a mixture of tri-*n*-butyltin chloride and the desired product. The solvent was then evaporated, and the residue was redissolved in ethyl ether and washed with 0.5 N HCl solution (2 × 50 mL), saturated potassium fluoride solution (2 × 50 mL), saturated sodium bicarbonate solution (2 × 50 mL), and brine. The organic layer was then dried ($MgSO_4$) and filtered, and the solvent was evaporated. Column chromatography (silica gel, neat hexanes followed by 10% ethyl acetate/hexanes) provided 2.21 g (54%) of pure vinyl ketone **4** as a white crystalline solid: mp 37–38 °C; IR (KBr) 2950, 2860, and 1710 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.34 (dd, J = 17.7, 10.3 Hz, 1H), 6.20 (dd, J = 17.7, 1.5 Hz, 1H), 5.81 (dd, J = 10.3, 1.5 Hz, 1H), 2.58 (t, J = 7.4 Hz, 2H), 2.38 (q, J = 7.0 Hz, 4H), 1.60 (m, 6H), 1.30 (m, 8H), 0.90 (t, J = 6.7 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 211.1, 200.5, 136.4, 127.8, 42.7, 42.2, 39.1, 31.4, 28.7, 28.6, 23.6, 23.4, 23.3, 22.3, 13.9; HRMS calcd for $C_{15}H_{26}O_2$ 238.1933, found 238.1924.

Benzyl-*N*-((*tert*-Butyloxycarbonyl)-*O*-benzyl-(*S*)-serine-hydroxamate (5). To a cold (0 °C) mixture of *O*-benzyl-*N*-(*tert*-butyloxycarbonyl)-(*S*)-serine (3.00 g, 10 mmol), triethylamine (6.5 mL, 4.72 g, 47 mmol), 1-hydroxybenzotriazole hydrate (2.10 g of 80%, 12.4 mmol), and *O*-benzylhydroxylamine hydrochloride (1.80 g, 11.0 mmol) in methylene chloride (100 mL) was added 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC, 2.95 g, 15.4 mmol). The reaction mixture was allowed to warm to room temperature and stir for an additional 24 h. Excess solvent was evaporated, and the residue was redissolved in ethyl acetate. The organic layer was washed with 0.5 N hydrochloric acid (2 × 50 mL) and saturated sodium bicarbonate (2 × 50 mL) and dried ($MgSO_4$), and the solvent was evaporated to give a pale yellow oil which was triturated with hexanes to give 3.64 g (89%) of a white solid: $[\alpha]^{25}_D$ +7.4 (c = 1, CH_2Cl_2); mp 78–79 °C; IR (KBr) 3320, 3270, 3150, 2980, 2870, 1665, 1530, 1495, and 1165 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.87 (br s, 1H), 7.36–7.31 (m, 10H), 5.30 (br s, 1H), 4.90 (s, 2H), 4.49 (AB q, J = 18.4, 11.7 Hz, 2H), 4.23 (br s, 1H), 3.84 (dd, J = 9.2, 4.2

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(11) Teng, M.; Miller, M. J. *J. Am. Chem. Soc.* **1993**, *115*, 548.

Hz, 1H), 3.50 (t, J = 8.2 Hz, 1H), 1.42 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 168.2, 155.3, 137.1, 135.0, 129.2, 128.7, 128.5, 128.5, 127.9, 127.7, 80.4, 78.2, 73.4, 69.4, 52.1, 28.2; HRMS calcd for $C_{22}H_{28}N_2O_5$ 400.1998; found: 400.2005.

***O,O*-Dibenzyl-*N*-(*tert*-butyloxycarbonyl)-(*S*)-norneoenactin A (6).** To a solution of the vinyl ketone **4** (0.50 g, 2.1 mmol) and (*S*)-serinehydroxamate **5** (0.85 g, 2.1 mmol) in benzene (50 mL) was added a catalytic amount of potassium *tert*-butoxide. The reaction mixture was refluxed for 36 h, after which time it was cooled to room temperature. Another equivalent of the vinyl ketone **4** was added, and the reaction mixture was refluxed for 4 days. Again, the reaction mixture was cooled to room temperature, another 0.5 equiv of **4** was added, and refluxing was continued for an additional 24 h. The solvent was evaporated, and the crude was chromatographed on silica gel (hexanes, 10% ethyl acetate/ hexanes, 30% ethyl acetate/hexanes) to afford 1.25 g (92%) of **6** as a viscous oil: $[\alpha]^{25}_D$ +5.6 (c = 1, CH_2Cl_2); IR (neat) 3430, 2930, 1710, 1660, 1490, 1450, and 1165 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.36–7.26 (m, 10H), 5.42 (d, 1H), 4.85 (s, 2H), 4.47 (AB q, J = 12.0, 2H), 4.11 (m, 1H), 3.77 (m, 1H), 3.69 (dd, J = 9.7, 5.0 Hz, 1H), 3.60 (dd, J = 9.6, 5.1 Hz, 2H), 2.58 (t, J = 6.7 Hz, 2H), 2.38–2.24 (m, 6H), 1.53 (m, 6H), 1.44 (s, 9H), 1.26 (m, 8H), 0.89 (t, J = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 211.1, 208.5, 171.4, 155.2, 137.6, 133.6, 129.5, 128.9, 128.6, 128.2, 127.5, 127.5, 79.6, 72.9, 69.8, 50.9, 42.7, 42.7, 42.2, 39.6, 31.5, 28.8, 28.5, 28.2, 23.7, 23.3, 23.0, 22.4, 13.9. Anal. Calcd for $C_{37}H_{54}N_2O_7$: C, 69.56; H, 8.52; N, 4.38. Found: C, 69.24; H, 8.18; N, 4.36.

***N*-(*tert*-Butyloxycarbonyl)-(*S*)-norneoenactin A (7).** To a solution of **6** (0.65 g, 1 mmol) in methanol (140 mL) was added 10% Pd/C (0.325 g, 0.3 mmol). The reaction mixture was stirred at room temperature and pressure under a hydrogen atmosphere for 11 h. The catalyst was filtered, the solvent evaporated, and the crude chromatographed on silica gel (3% methanol/methylene chloride) to give 0.37 g (80%) of a white solid which was recrystallized from ethyl ether to give **7** as a white crystalline solid: $[\alpha]^{25}_D$ +4.2 (c = 1, CH_2Cl_2); mp 88–89.5 °C; IR (KBr) 3420, 2980, 1705, 1690, 1590, 1525, and 1470 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 9.33 (br s, 1H), 5.78 (br d, 1H), 4.78 (br s, 1H), 3.94–3.71 (m, 4H), 2.76 (t, 2H), 2.47–2.34 (m, 6H), 1.53 (quintet, J = 6.9 Hz, 6H), 1.42 (s, 9H), 1.25 (m, 8H), 0.86 (t, J = 6.1 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 211.5, 210.5, 170.0, 156.8, 80.8, 63.3, 50.5, 43.3, 42.9, 42.5, 42.3, 39.7, 31.6, 28.9, 28.5, 28.3, 23.8, 23.3, 23.2, 22.5, 14.0. Anal. Calcd for $C_{23}H_{42}N_2O_7$: C, 60.24; H, 9.23; N, 6.11. Found: C, 60.25; H, 8.87; N, 6.18.

Norneoenactin A Sulfate [(*S*)-1)]. A solution of **7** (86.4 mg, 0.2 mmol) in methylene chloride/trifluoroacetic acid (1:1, 8 mL) was stirred at room temperature for 0.5 h. The volatiles were then evaporated, and the resulting colorless oil was redissolved in methylene chloride (10 mL). A dilute solution of sulfuric acid (0.2 M, 10 mL) was added, and the resulting reaction mixture was stirred at room temperature for 1 h during which time a white solid precipitated. The reaction mixture was then cooled to ~5 °C overnight. The resulting white solid was filtered to give 62.8 mg (82%) of (*S*)-**1**: mp 112 °C dec; IR (KBr) 3420, 2930, 1700, 1625, and 1480 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 4.50–3.47 (m, 5H), 2.68 (m, 2H), 2.37 (t, J = 7.2 Hz, 6H), 1.42 (quintet, J = 7.1 Hz, 6H), 1.21 (m, 8H), 0.84 (t, J = 6.7 Hz, 3H); FAB (MH^+) m/e 359 (calcd 359). Anal. Calcd for $C_{18}H_{34}N_2O_5 \cdot 1/2H_2O \cdot 1/2H_2SO_4$: C, 51.91; H, 8.71; N, 6.73. Found: C, 51.64; H, 8.31; N, 6.62.

***O*⁴-Benzyl-*N*-(*tert*-butyloxycarbonyl)-(*R*)-norneoenactin A (9).** A catalytic amount of potassium *tert*-butoxide was added to a solution of the vinyl ketone **4** (0.21 g, 0.9 mmol) and (*R*)-serinehydroxamate **8** (0.25 g, 0.8 mmol) in 45 mL of dioxane. The reaction mixture was refluxed for 19 h, after which time it was cooled to room temperature. Another 0.5 equiv of the vinyl ketone **4** was added, and the reaction mixture was refluxed for an additional 20 h. Excess solvent was evaporated, and the crude residue was chromatographed on silica gel (50% ethyl acetate/ hexanes) to afford 249 mg (56%) of product **9** as a viscous oil: $[\alpha]^{25}_D$ −9.3 (c = 1, CH_2Cl_2); IR (neat) 3430, 2930, 1705, 1655, and 1055 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.40–7.20 (m, 5H), 5.57 (d, 1H), 4.90 (s, 2H), 4.79–4.72 (m, 1H), 4.32–4.22 (m, 1H), 3.85–3.58 (m, 4H), 2.66 (t, J = 6.41 Hz, 2H), 2.44–2.32 (m, 6H), 1.59–1.49 (m, 6H), 1.45 (s, 9H), 1.33–1.16 (m, 8H), 0.88 (t, J = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 213.9, 211.7, 173.4, 157.1, 134.6, 130.5, 130.0, 129.6, 79.8, 76.7, 62.7, 52.4, 42.1, 42.0, 41.6, 39.8, 38.5, 30.6, 27.9, 27.5, 27.3, 22.7, 22.2, 22.0, 21.3, 12.7. Anal. Calcd for $C_{37}H_{54}N_2O_7$: C, 65.67; H, 8.82; N, 5.11. Found: C, 65.82; H, 8.73; N, 5.05.

***N*-(*tert*-Butyloxycarbonyl)-(*R*)-norneoenactin A (10).** To a solution of **9** (0.11 g, 0.2 mmol) in ethanol (25 mL) was added 10% Pd/C (0.060 g, 56 μmol). The reaction mixture was stirred at room temperature and pressure under a hydrogen atmosphere for 105 min. The catalyst was removed by filtration through Celite, and the solvent was evaporated. The crude residue was chromatographed on silica gel (3% methanol/methylene chloride) to afford 47 mg (51%) of a white solid which was recrystallized from ethyl ether to give **10** as a white crystalline solid: mp 88–89 °C; ^{1}H NMR ($CDCl_3$) δ 9.33 (br s, 1H), 5.82 (br d, 1H), 5.02–4.78 (m, 1H), 3.98–3.71 (m, 4H), 2.78 (t, 2H), 2.50–2.33 (m, 6H), 1.62–1.50 (m, 6H), 1.44 (s, 9H), 1.34–1.20 (m, 8H), 0.88 (t, J = 6.5 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 211.6, 210.4, 169.9, 156.6, 80.6, 63.2, 51.2, 43.4, 42.8, 42.5, 42.3, 39.6, 31.5, 28.9, 28.5, 28.2, 23.8, 23.3, 23.2, 22.4, 14.0. Anal. Calcd for $C_{23}H_{42}N_2O_7$: C, 60.24; H, 9.23; N, 6.11. Found: C, 60.20; H, 9.42; N, 6.13.

Norneoenactin A Sulfate [(*R*)-1)]. A solution of **10** (40.0 mg, 87 μmol) in methylene chloride/trifluoroacetic acid (1:1, 10 mL) was stirred at room temperature for 1 h. The volatiles were then evaporated, and the resulting colorless oil was redissolved in methylene chloride (5 mL). A dilute solution of sulfuric acid (0.2 M, 5 mL) was added, and the resulting reaction mixture was stirred at room temperature for 1 h, during which time a white solid precipitated. The reaction mixture was then cooled to ~5 °C overnight. The resulting white solid was filtered and air dried to give 29 mg (82%) of (*R*)-**1**: mp 112 °C dec; ^{1}H NMR (DMSO-d_6) δ 4.50–3.50 (m, 5H), 2.68 (m, 2H), 2.58–2.41 (m, 2H), 2.37 (t, J = 7.3 Hz, 4H), 1.42 (quintet, J = 7.3 Hz, 6H), 1.21 (m, 8H), 0.84 (t, J = 6.8 Hz, 3H). Anal. Calcd for $C_{18}H_{34}N_2O_5 \cdot 1/2H_2SO_4$: C, 53.05; H, 8.66; N, 6.87. Found: C, 52.69; H, 8.32; N, 6.82.

OPA-HPLC analysis of (*R*)-1 and (*S*)-1. In a 500-μL disposable microcentrifuge tube was added 10 μL of a 2 mM solution of (*R*)-**1**, (*S*)-**1**, or a mixture of the two enantiomers in 1:1 MeOH/H_2O followed by 10 μL of derivatization reagent. (The derivatization reagent was made by dissolving 4 mg of *o*-phthalicdicarboxaldehyde (OPA) in 300 μL of methanol followed by addition of 250 μL of a 0.4 M solution of sodium borate buffer (pH 9.5), 390 μL of DDH_2O, and 60 μL of a 1 M solution of *N*-acetylcysteine (NAC)). After 2 min, 40 μL of 0.1 M potassium dihydrogen phosphate was added to quench the reaction. A 25-μL aliquot was injected into the HPLC column. An isocratic solution of 1:19:80 of THF/MeOH/NaOAc buffer (pH 5.8) was used as the eluting solvent at a flow rate of 1.0 mL/min. The enantiomeric purities were determined to be >98% (>96% ee) for (*S*)-**1** and 93% (86% ee) for (*R*)-**1**.

Acknowledgment. The financial support of the National Institute of Health is gratefully appreciated. We also sincerely appreciate the preliminary biological tests performed at Eli Lilly Co., Indianapolis, IN.