Synthesis of the Antifungal Agent Neoenactin A and Its *N*-Deshydroxy Derivative

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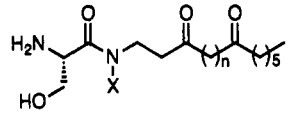
The first total and unequivocal syntheses of neoenactin A (1, NE A) as well as its *N*-deshydroxy analog 2 are described. The two key steps in the syntheses were the oxidative cleavage of an appropriate olefin 8a to yield the desired keto acid 9 and the Michael addition of serinehydroxamates 11 or 12 to the appropriate vinyl ketone 10. Biological activity of 1 and 2 against *Escherichia coli* and several fungi has been investigated.

Introduction

Neoenactins (NEs) are a new family of L-serinehydroxamic acid-based antifungal agents produced by *Streptomyces roseoviridis*.[1] The potency of these compounds against a wide variety of fungi has been demonstrated.[1b] 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, 1), and analogs. We have already reported[3] the synthesis of norneoenactin A (3), an analog of NE A, and herein we describe the first total syntheses of NE A (1) as well as *N*-deshydroxyneoenactin A (2).



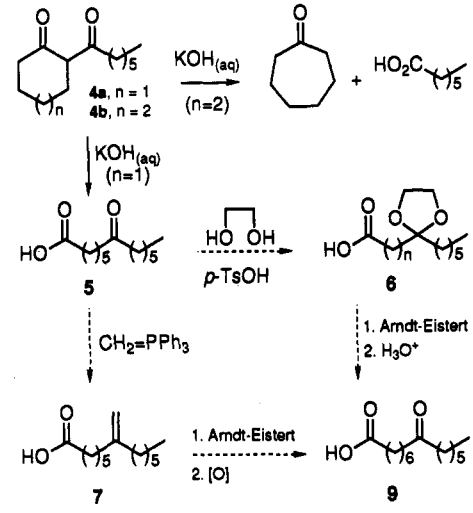
1, Neoenactin A (NE A), n=6, X=OH
2, *N*-Deshydroxy-neoenactin A, n=6, X=H
3, Norneoenactin A, n=5, X=OH

Results and Discussion

To synthesize neoenactin A, we wanted to develop a flexible convergent approach which should provide NE A (1) in addition to a number of analogs. On the basis of our earlier work,[3] Michael addition of the hydroxamate nitrogen of serinehydroxamates 11 or 12 to vinyl ketone 10 should, upon further manipulation, provide the target molecule 1.

In our successful attempt to synthesize norneoenactin A (3),[3] we have reported the synthesis of 7-oxotridecanoic acid (5), which was obtained by basic hydrolysis of 2-heptanoylcyclohexanone (4a, Scheme 1). Unfortunately, 8-oxotetradecanoic acid (9) could not be obtained by basic hydrolysis of 2-heptanoylcycloheptanone (4b).[4] Interestingly, simple variation from cyclohexanone 4a to cycloheptanone 4b drastically alters the regioselectivity of nucleophilic attack on these diones. This is attributed to the decreased activity of the ring carbonyl group caused by a shielding effect due to ring puckering. Thus, base

Scheme 1



hydrolysis of 2-heptanoylcycloheptanone (4b) provides cycloheptanone and hexanoic acid as the main products.

A literature search revealed three reports for the synthesis of keto acid 9, the direct precursor of vinyl ketone 10. In the first,[5] the acid chloride of suberic acid monomethyl ester was treated with 1-hexylcadmium chloride to give the methyl ester of 9 which upon base hydrolysis afforded 9 in 63% overall yield. Although the starting suberic acid monoethyl ester is commercially available, its use would not have allowed flexibility for the eventual preparation of analogs. The other two references were not readily available.[6]

Alternatively, it should be possible to obtain 8-oxotetradecanoic acid (9) from 7-oxotridecanoic acid (5) employing one of two approaches. First, conversion of ketone 5[3] to ketal 6, homologation utilizing the Arndt–Eistert protocol, and acid hydrolysis should provide keto acid 9. Conversion of the keto group in 5 to olefin 7, homologation, and oxidative cleavage of the double bond is yet another route that could provide the desired keto acid 9. However, initial difficulties in isolating or purifying the ketal acid 6, in addition to the numerous steps required to protect, homologate, and deprotect the ketone in 5, led us to seek an alternative, more flexible route to neoenactin A (1) and analogs.

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

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

(3) Darwish, I. S.; Patel, C.; Miller, M. J. *J. Org. Chem.* **1993**, *58*, 6072.

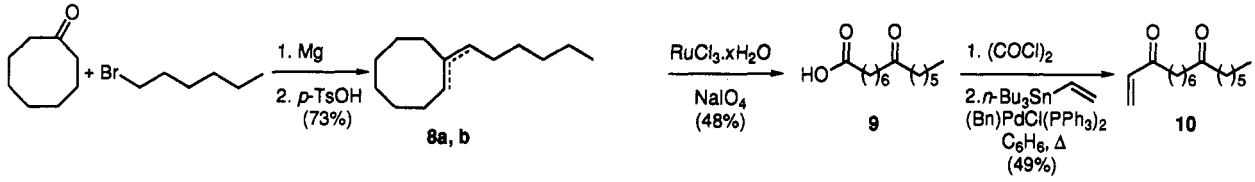
(4) Kawamura, K.; Gagosian, R. B. *J. Chromatogr.* **1988**, *438*, 299.

(5) Aragozzini, F.; Manachini, P. L.; Craveri, R.; Rindone, B.; Scolastico, C. *Tetrahedron* **1972**, *28*, 5493.

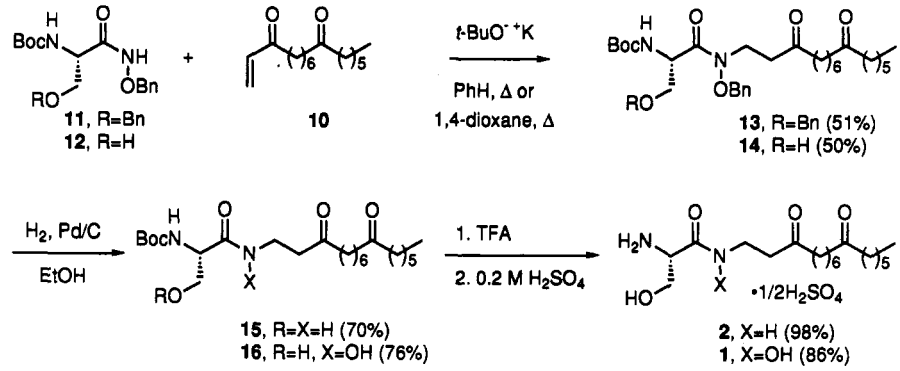
(6) (a) Breusch, F. L.; Kirkal, A. *Fette, Seifen, Anstrichmittel* **1963**, *65*, 995. (b) Faqqua, S. A. Ph.D. Thesis, University of Wisconsin at Madison 1957.

0022-3263/94/1959-0451$04.50/0

Scheme 2



Scheme 3



Olefins, which can be considered as disguised aldehydes or ketones, are readily converted to aldehydes or ketones by oxidation. Therefore, an appropriately substituted olefin should provide the desired keto acid **9** upon oxidative cleavage of the double bond. In the new approach, cyclooctanone and 1-bromohexane, which are commercially available (Aldrich) and very inexpensive, were the starting materials. Treatment of 1-bromohexane with magnesium turnings[7] provided the Grignard reagent, 1-hexylmagnesium bromide (Scheme 2). Addition of a solution of cyclooctanone in tetrahydrofuran to the Grignard reagent provided the tertiary alcohol after workup. The tertiary alcohol was not isolated but was immediately dehydrated in the presence of *p*-toluenesulfonic acid in refluxing benzene (azeotropic removal of water using Dean–Stark apparatus) to provide moderate yields of a 95:5 mixture of the two isomeric olefins **8a** and **8b**. Synthesis of 1-hexylcyclooctene (**8a**) was briefly mentioned in one report without disclosure of any physical data.[8]

Since the two isomers of **8** are very similar and inseparable, it was not possible to assign which isomer was the major product. However, this problem was readily solved by carrying out the oxidation reaction. Treatment of the isomeric mixture with a catalytic amount of ruthenium(III) chloride hydrate in the presence of sodium periodate in a solution of carbon tetrachloride, acetonitrile, and water provided the desired 8-oxotetradecanoic acid (**9**) in moderate yields, demonstrating that the major isomer was the desired 1-hexylcyclooctene (**8a**).

Since an adequate amount of **9** was secured, conversion to the desired vinyl ketone **10** was then attempted (Scheme 2). Treatment of **9** with oxalyl chloride provided the acid chloride, which was immediately converted to vinyl ketone **10** upon refluxing with vinyltri-*n*-butylstannane in the presence of a catalytic amount of *trans*-benzyl(chloro)bistriphenylphosphinepalladium(II).

With the successful synthesis of **10**, we then carried out the Michael addition of benzyl *N*-(*tert*-butyloxycarbonyl)-*O*-benzyl-(*S*)-serinehydroxamate (**11**)[9] with vinyl ketone **10**, in the presence of a catalytic amount of potassium *tert*-butoxide (Scheme 3), to give a moderate yield of desired adduct **13**. Catalytic hydrogenation of the benzyl groups in **13** using palladium on carbon, however, provided an inseparable mixture of the desired debenzylated product as well as the N^4-deshydroxy compound. Reduction of the N–O bond was concomitant with reduction of the benzyl ether functionality. Various manipulations of the reaction conditions including changing the solvent, mole equivalents of palladium, and reaction time proved unsuccessful. We therefore decided to use the overreduction to our advantage and completely reduce the N–O bond, thus obtaining a deshydroxy analog of NE A to determine whether the hydroxamate hydroxyl group is essential for biological activity. Thus, performing the reduction for an extended period of time provided **15** in 70% yield. Finally, removal of the Boc group using TFA followed by treatment of the triflate salt with a 1:1 mixture of methylene chloride and dilute sulfuric acid provided the deshydroxy analog of NE A, N^4-deshydroxyneoenactin A sulfate (**2**), as a white precipitate.

To synthesize neoenactin A itself, the Michael reaction was carried out using benzyl *N*-(*tert*-butyloxycarbonyl)-(*S*)-serinehydroxamate (**12**, Scheme 3),[10] instead of the bisbenzylated serinehydroxamate **11**, to give monobenzylated product **14** in 50% yield. Catalytic reduction of **14** (Pd/C) provided a good yield of the desired Boc-neoenactin A (**16**). Deprotection of **16** (TFA) followed by conversion to the sulfate salt provided neoenactin A sulfate (**1**) as a white crystalline solid.

The two compounds, **1** and **2**, were tested against *E. coli* and a select number of fungi, including *Candida albicans*, *Aspergillus fumigatus*, and *Cryptococcus neoformans*. Although neoenactin A sulfate (**1**) and N^4-deshydroxy-neoenactin A sulfate (**2**) were inactive against *E. coli*, neoenactin A sulfate (**1**) exhibited significant inhibitory activity against *Candida albicans* (MIC 0.625 μg/mL) and *Cryptococcus neoformans* (MIC 0.156 μg/mL) and mod-

(7) The turnings were washed with 1 N HCl, water, methanol, and ether and oven dried.
(8) Just, G.; Payette, D. R. *Tetrahedron Lett.* **1980**, *21*, 3219.
(9) Ramasamy, K.; Olsen, R. K.; Emery, T. *J. Org. Chem.* **1981**, *46*, 5438 (no experimental or physical data were reported).
(10) Miller, M. J.; Mattingly, P. G.; Morrison, M. A.; Kerwin, J. F., Jr. *J. Am. Chem. Soc.* **1980**, *102*, 7026.

erate inhibitory activity against *Aspergillus fumigatus* (MIC 20 μg/mL). On the other hand, compound **2** was active against *Cryptococcus neoformans* (MIC 2.5 μg/mL) but failed to inhibit *Candida albicans* and *Aspergillus fumigatus* (MIC 20 and 80 μg/mL, respectively).[11] 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.[12]

1-Hexylcyclooctene (8a).[8] A flame-dried, round-bottomed flask was charged with magnesium (15.4 g, 0.6 mol), a crystal of iodine, and anhydrous THF (25 mL) under argon. Neat 1-bromohexane (45 mL, 52.9 g, 0.32 mol) was added dropwise while the reaction mixture was cooled slightly. After 15 min, a solution of cyclooctanone (9.93 g, 78.7 mmol, previously dried by azeotroping with benzene) in 25 mL of anhydrous THF was added slowly while the reaction vessel was cooled (ice bath). The resulting reaction mixture was stirred at rt under argon overnight after which time it was poured over crushed ice and stirred for 5 min. A 10% solution of HCl was added slowly until bubbling stopped. The two layers were then separated, and the aqueous layer was washed with ether (3 × 50 mL). The organic layers were combined, and the solvent was evaporated to give an oily residue, which was redissolved in benzene (65 mL). *p*-Toluenesulfonic acid (catalytic amount) was added, and the resulting reaction mixture was refluxed, using a Dean-Stark apparatus, overnight. The reaction mixture was then cooled to rt and washed with saturated $NaHCO_3$ solution (2 × 50 mL) and brine (2 × 50 mL). The organic layer was then separated, dried ($MgSO_4$), and filtered, and the solvent was evaporated to give 25 g of a yellow crude oil. Column chromatography on silica gel (neat hexanes) provided 11.1 g (73%) of a colorless oil: IR (neat) 2925, 2850, 1465, and 1490 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.31 (major isomer, t, J = 8.1 Hz, 1H), 5.15 (minor isomer, t, J = 7.3 Hz, 1H), 2.20–2.03 (m, 4H), 1.96 (t, J = 7.6 Hz, 2H), 1.65-1.20 (m, 16H), 0.88 (t, J = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 141.1, 123.4, 37.7, 31.9, 30.0, 29.3, 28.93, 28.91, 28.2, 26.5, 26.3, 26.3, 22.7, 14.1; HRMS (MH^+) calcd for $C_{14}H_{26}$ 194.2034, found 194.2023. Anal. Calcd for $C_{14}H_{26}$: C, 86.52; H, 13.48. Found: C, 86.56; H, 13.40.

8-Oxotetradecanoic Acid (9).[5,6] A solution of olefins **8a** and **8b** (10.0 g, 51 mmol), $RuCl_3.H_2O$ (1.25 g, 6 mmol), and $NaIO_4$ (34.16 g, 160 mmol) in a mixture of CH_3CN (100 mL), CCl_4 (100 mL), and H_2O (300 mL) was stirred at rt for 7.5 d. Ethyl acetate (300 mL) and brine (300 mL) were added to the above solution, and the two phase reaction mixture was filtered through a pad of Celite to give a yellow two-phase solution. The aqueous layer was separated and extracted with EtOAc (3 × 100 mL) after which time the organic layers were combined, washed with brine (1 × 100 mL), dried ($MgSO_4$), filtered, and the solvent was evaporated to give a gray solid. Column chromatography on silica gel (50% EtOAc/hexanes) provided 5.9 g (48%) of a white solid: mp 60–61.5 °C (lit.[6] mp 69–70 °C (corrected)); IR (KBr) 3125, 2930, 2650, and 1695 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.45 (m, 6H), 1.69–1.45 (m, 6H), 1.41–1.20 (m, 10H), 0.88 (t, J = 6.7 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 211.7, 179.8, 42.8, 42.6, 33.9, 31.6, 28.9, 28.8, 24.4, 23.8, 23.5, 22.4, 14.0; HRMS (MH^+) calcd for $C_{14}H_{26}O_3$ 242.1881, found 242.1875. Anal. Calcd for $C_{14}H_{26}O_3$: C, 69.38; H, 10.81; O,19.80. Found: C, 69.58; H, 10.82; O, 19.63.

3,10-Dioxohexadec-1-ene (10). To a solution of acid **9** (4.35 g, 18 mmol) in 75 mL of CH_2Cl_2 was added oxalyl chloride (3.5 mL, 5.1 g, 40 mmol). The reaction mixture was then refluxed for 2 h and cooled to rt, and the volatiles were removed *in vacuo*. The resulting residue was redissolved in benzene (75 mL), and vinyltri-*n*-butylstannane (Fluka, 5.2 mL, 5.64 g, 17.8 mmol) and a catalytic amount of benzyl(chloro)bis(triphenylphosphine)-palladium(II) were added. The resulting reaction mixture was refluxed for 2.5 h after which time it was cooled to rt and the solvent was evaporated. The black residue was then dissolved in ethyl ether and washed with 0.5 N HCl solution (2 × 50 mL), saturated KF solution (2 × 50 mL), saturated $NaHCO_3$ 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, 10% ethyl acetate/hexanes) provided 2.21 g (49%) of pure vinyl ketone **10** as a white crystalline solid: mp 37–38 °C; ^1H NMR ($CDCl_3$) δ 6.35 (dd, J = 17.8, 10.3 Hz, 1H), 6.21 (dd, J = 17.7, 1.5 Hz, 1H), 5.82 (dd, J = 10.3, 1.5 Hz, 1H), 2.58 (t, J = 7.4 Hz, 2H), 2.39 (t, J = 7.3 Hz, 2H), 2.38 (t, J = 7.4 Hz, 2H), 1.67–1.50 (m, 6H), 1.37–1.21 (m, 10H), 0.88 (t, J = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 211.4, 200.8, 136.5, 127.8, 42.7, 42.5, 39.4, 31.5, 28.9, 28.8, 23.7, 23.7, 23.5, 22.4, 13.9; HRMS (MH^+) calcd for $C_{16}H_{28}O_2$ 253.2168, found 253.2167. Anal. Calcd for $C_{16}H_{28}O_2$: C, 76.14; H, 11.18. Found: C, 75.85; H, 11.25.

***O,O*-Dibenzyl-*N*-(*tert*-butyloxycarbonyl)neoenactin A (13).** To a solution of the vinyl ketone **10** (0.85 g, 2.1 mmol) and (*S*)-serinehydroxamate **11**[3,9] (0.50 g, 2.0 mmol) in toluene (50 mL) was added a catalytic amount of potassium *tert*-butoxide. The reaction mixture was refluxed under argon for 9 h after which time it was cooled to rt. Another 0.25 g (1 mmol) of vinyl ketone **10** was added, and the reaction mixture was refluxed for 30 h. Again, the reaction mixture was cooled to rt, another 0.10 g (0.4 mmol) of **10** was added, and refluxing was continued for an additional 24 h. The volatiles were evaporated, and the crude product residue was chromatographed on silica gel (25% EtOAc/hexanes) to afford 0.71 g (51%) of **13** as a viscous oil: $[\alpha]^{25}_D$ +6.2 (c = 1, CH_2Cl_2); IR (neat) 3440, 3340, 2940, 2760, 1710, 1665, 1490, 1450, and 1365 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.39–7.20 (m, 10H), 5.44 (br d, 1H), 4.95 (m, 1H), 4.85 (s, 2H), 4.47 (q, J = 28.9, 12.1 Hz, 2H), 4.20–4.08 (m, 1H), 3.83–3.58 (m, 3H), 2.58 (t, J = 6.7 Hz, 2H), 2.40–2.24 (m, 6H), 1.60–1.48 (m, 6H), 1.45 (s, 9H), 1.31–1.15 (m, 10H), 0.88 (t, J = 6.7 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 211.4, 208.8, 171.5, 155.3, 137.7, 133.7, 129.5, 129.0, 128.6, 128.3, 127.6, 127.6, 79.7, 73.0, 69.8, 51.0, 43.0, 42.8, 42.6, 40.6, 39.6, 31.6, 28.9, 28.9, 28.8, 28.3, 23.8, 23.5, 23.2, 22.5, 14.0; HRMS (MH^+) calcd for $C_{38}H_{56}N_2O_7$ 653.4166, found 653.4185. Anal. Calcd for $C_{38}H_{56}N_2O_7$: C, 69.91; H, 8.65; N, 4.29. Found: C, 70.06; H, 8.86; N, 4.20.

***O*⁴-Benzyl-*N*-(*tert*-butyloxycarbonyl)neoenactin A (14).** To a solution of the vinyl ketone **10** (0.65 g, 2.6 mmol) and (*S*)-serinehydroxamate **12**[10] (0.67 g, 2.2 mmol) in 1,4-dioxane (50 mL) was added a catalytic amount of potassium *tert*-butoxide (50 mg, 0.4 mmol). The resulting reaction mixture was refluxed under argon for 24 h after which time it was cooled to rt. Another 0.10 g of vinyl ketone **10** was added, and the reaction mixture was refluxed for 5 d. The reaction mixture was cooled to rt, another 0.18 g of **10** was added, and refluxing was continued for an additional 21 h. The volatiles were evaporated, and the crude was chromatographed on silica gel (2% MeOH/CH_2Cl_2) to afford 0.58 g (50%) of **14** as a viscous oil: $[\alpha]^{25}_D$ +14.7 (c = 1, CH_2Cl_2); IR (KBr) 3500, 3340, 2940, 1705, 1678, and 1567 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.41–7.37 (m, 5H), 5.57 (br d, J = 8.3 Hz, 1H), 4.90 (s, 2H), 4.80–4.73 (m, 1H), 4.33–4.20 (m, 1H), 3.85–3.58 (m, 3H), 2.67 (t, J = 6.4 Hz, 2H), 2.42–2.34 (m, 6H), 1.57–1.47 (m, 6H), 1.45 (s, 9H), 1.31–1.21 (m, 10H), 0.88 (t, J = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 211.5, 209.3, 171.6, 155.7, 133.6, 129.5, 129.1, 128.7, 79.8, 76.8, 63.2, 52.9, 42.9, 42.8, 42.5, 40.5, 39.2, 31.5, 28.8, 28.7, 28.3, 23.7, 23.5, 23.2, 22.4, 14.0; HRMS (MH^+) calcd for $C_{31}H_{50}N_2O_7$ 563.3696, found 563.3696. Anal. Calcd for $C_{31}H_{50}N_2O_7$: C, 66.16; H, 8.96; N, 4.98. Found: C, 66.38; H, 8.96; N, 4.86.

***N*-(*tert*-Butyloxycarbonyl)-*N*⁴-deshydroxyneoenactin A (15).** To a solution of the bisbenzylated Boc-neoenactin A **13** (100 mg, 0.15 mmol) in ethanol (20 mL) was added 10% Pd/C (50 mg, 47 μmol). The reaction mixture was stirred at rt and atmospheric pressure under a hydrogen atmosphere for 7 d. The solution was filtered through Celite to give a white solid which was recrystallized from ethyl ether to give 49 mg (70%) of a white crystalline solid: mp 71–73 °C; ^1H NMR ($CDCl_3$) δ 7.00 (br s, 1H), 5.53 (br s, 1H), 4.18–3.95 (m, 2H), 3.75–3.70 (br s, 1H), 3.50 (AB q, J = 5.8 Hz, 2H), 3.21–3.00 (m, 1H), 2.64 (t, J = 5.8 Hz, 2H), 2.42–2.36 (m, 6H), 1.60–1.50 (m, 6H), 1.45 (s, 9H), 1.33–1.23 (m, 10H), 0.88 (t, J = 6.5 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 211.7, 210.1, 171.2, 155.9, 80.4, 62.9, 55.2, 42.8, 42.8, 42.5, 41.8, 34.1, 31.6, 29.7, 28.9, 28.2, 23.8, 23.5, 23.4, 22.5, 14.0. Anal. Calcd for $C_{24}H_{44}N_2O_6$: C, 63.13; H, 9.71; N, 6.13. Found: C, 63.46; H, 9.39; N, 5.95.

(11) Biological data at elevated concentrations were obtained at Eli Lilly and Co., Indianapolis, IN.

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

solution was then filtered through Celite to give a white solid which was chromatographed on silica gel (3% MeOH/CH_2Cl_2) to give 318 mg (76%) of a white crystalline solid: mp 78.5–80.0 °C; $[\alpha]^{25}_D$ +2.0 (c = 1, CH_2Cl_2); IR (KBr) 3460, 3350, 3160, 2935, 1705, 1685, 1590, and 1535 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.35 (br s, 1H), 5.95–5.65 (m, 1H), 4.86–4.61 (m, 1H), 4.25–3.53 (m, 4H), 2.82–2.75 (m, 2H), 2.46 (t, J = 7.4 Hz, 2H), 2.39 (t, J = 7.4 Hz, 4H), 1.61–1.48 (m, 6H), 1.44 (s, 9H), 1.33–1.23 (m, 10H), 0.88 (t, J = 6.7 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 214.2, 213.1, 171.6, 158.1, 80.8, 63.0, 50.5, 42.7, 42.2, 42.1, 41.9, 38.9, 30.7, 28.0, 27.9, 27.3, 22.8, 22.5, 22.3, 21.4, 12.8; HRMS (MH^+) calcd for $C_{24}H_{44}N_2O_7$ 473.3227, found 473.3229. Anal. Calcd for $C_{24}H_{44}N_2O_7$: C, 60.99; H, 9.38; N, 5.93. Found: C, 60.77; H, 9.37; N, 5.85.

N^3-Deshydroxyneoenactin A Sulfate (2). A solution of **15** (63 mg, 0.14 mmol) in CH_2Cl_2/TFA (1:1, 8 mL) was stirred at rt for 1 h. The volatiles were then evaporated, and the resulting colorless oil was redissolved in CH_2Cl_2 (10 mL). A dilute solution of sulfuric acid (0.2 M, 10 mL) was added, and the resulting reaction mixture was stirred at rt for 1 h during which time a white solid precipitated. The reaction mixture was then cooled to ~5 °C overnight, and the resulting white solid was filtered to give 55 mg (98%) of **2**: mp 131–133 °C; IR (KBr) 3400, 2960, 1720, and 1665 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.17 (m, 1H), 3.63–3.21 (m, 8H), 2.58 (t, J = 6.6 Hz, 2H), 2.38 (AB q, J = 7.0 Hz, 6H), 1.50–1.35 (m, 6H), 1.30–1.15 (m, 10H), 0.84 (t, J = 6.7 Hz, 3H); HRMS (MH^+) calcd for $C_{19}H_{36}N_2O_4$ 357.2753, found 357.2758. Anal. Calcd for $C_{19}H_{36}N_2O_4 \cdot 1/2H_2O \cdot 1/2H_2SO_4$: C, 55.05; H, 9.24; N, 6.76. Found: C, 54.80; H, 8.81; N, 6.44.

Neoenactin A Sulfate (1). A solution of **16** (50 mg, 0.1 mmol) in CH_2Cl_2/TFA (1:1, 10 mL) was stirred at rt for 1.5 h. The volatiles were then evaporated, and the resulting colorless oil was redissolved in CH_2Cl_2 (5 mL). A dilute solution of sulfuric acid (0.2 M, 5 mL) was added, and the resulting reaction mixture was stirred at rt for 1 h during which time a white solid precipitated. The reaction mixture was then cooled to ~5 °C overnight and the resulting white solid was filtered to give 34 mg (86%) of **1**: mp 151 °C dec; IR (KBr) 3440, 3140, 2940, 1705, 1628, and 1480 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.75 (br s, 1H), 4.48–3.44 (m, 8H), 2.73–2.55 (m, 2H), 2.37 (t, J = 7.3 Hz, 6H), 1.47–1.13 (m, 16H), 0.84 (t, J = 6.7 Hz, 3H); HRMS (MH^+) calcd for $C_{19}H_{36}N_2O_5$ 373.2702, found 373.2688. Anal. Calcd for $C_{19}H_{36}N_2O_5 \cdot 1/2H_2SO_4$: C, 54.14; H, 8.85; N, 6.65. Found: C, 53.87; H, 8.83; N, 6.33.

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