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Asymmetric Synthesis of (+)-Negamycin

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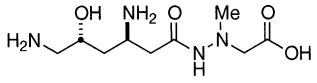
Received February 20, 2002

An asymmetric synthesis of the antibiotic (+)-negamycin (**1**) has been achieved, starting from commercially available (5*R*,6*S*)-4-(benzyloxycarbonyl)-5,6-diphenyl-2,3,5,6-tetrahydro-4*H*-1,4-oxazin-2-one (**2**). The synthesis involved the stabilized Wittig olefination of the lactone carbonyl group of **2** and subsequent asymmetric hydrogenation to generate the corresponding all-*syn* oxazine **4** with excellent diastereoselectivity. Conversion of **4** into *β*-alkoxy imine **7** and subsequent $CeCl_3$-promoted chelation-controlled allylation of **7** generated the corresponding homoallylamine **8** with good diatereoselectivity, which was readily converted into (+)-negamycin (**1**) in 25% overall yield over 11 steps.

Introduction

(+)-Negamycin (**1**, 2-[(3*R*,5*R*)-3,6-diamino-5-hydroxyhexanoyl]-1-methylhydrazinoacetic acid) (Figure 1) is an unusual antibiotic containing a hydrazido peptide linkage.[1] First isolated in 1970 by Umezawa and co-workers[2] from culture filtrates of three strains closely related to *Streptomyces purpeofuscus*, the structure of negamycin was confirmed in 1972 by total synthesis from D-galacturonic acid.[3] Negamycin exhibits considerable inhibitory activity toward multiple drug-resistant enteric Gram-positive and Gram-negative bacteria and exhibits low acute toxicity.[2] Negamycin is a specific inhibitor of protein biosynthesis with miscoding activity.[4]

A number of approaches have been reported in the literature for the synthesis of racemic[5] as well as optically active[6] negamycin. Most of these approaches relied on C5 and/or C3 stereogenic centers of negamycin preexisting in the starting materials.[6a,d−g,i−j] The synthesis



(+)-Negamycin (1)

FIGURE 1.

of optically active negamycin described in these literature procedures required 8−20 steps, with yields ranging between 7 and 32%.[6] Herein, we report a concise method for the synthesis of (+)-negamycin by employing (5*R*,6*S*)-4-(benzyloxycarbonyl)-5,6-diphenyl-2,3,5,6-tetrahydro-4*H*-1,4-oxazin-2-one (**2**)[7] as a starting material. Our approach involves the generation of the C5 and C3 stereogenic centers of negamycin by employing the asymmetric hydrogenation of dihydrooxazine derivative **3** and a chelation-controlled allylation of *β*-alkoxy imine **7**, respectively.

Results and Discussion

As shown in Scheme 1, the Wittig reaction of methyl (triphenylphosphoranylidene)acetate with the lactone carbonyl group[8] of **2** (xylene, 210 °C, 2 h) generated the adduct **3** in quantitative yield. This species is reasonably presumed to arise via tautomerization of the initial olefination product ***A*** to the thermodynamically more stable trisubstituted olefin. The stabilized Wittig olefination of **2** has previously been studied by our group and was applied to the asymmetric synthesis of (+)-hypusine.[9]

* Corresponding author.

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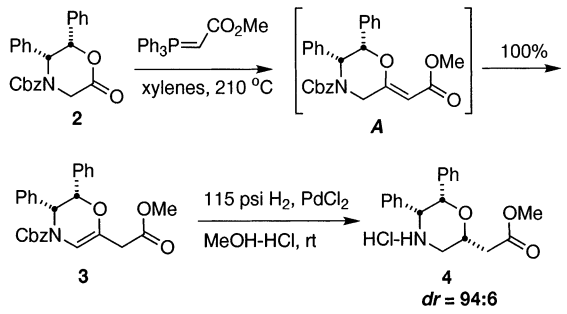
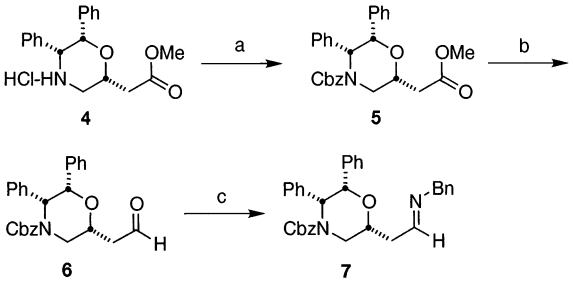
10.1021/jo025636i CCC: $22.00
Published on Web 08/02/2002

SCHEME 1



SCHEME 2[a]



[a] Reagents and conditions: (a) Cbz−Cl, Et_3N, DMAP, CH_2Cl_2, rt, 96%; (b) DIBALH, CH_2Cl_2, −78 °C, 85%; (c) $BnNH_2$, Al_2O_3, CH_2Cl_2, 0 °C, 98%.

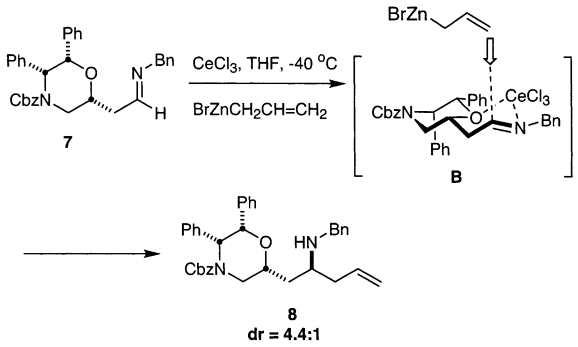
Application of compound **3** (as the corresponding ethyl ester) as a key precursor in the synthesis of (*R*)-(−)-carnitine has also been reported from this laboratory.[10]

Hydrogenation of **3** with $PdCl_2$ (20 mol %, 115 psi of H_2, MeOH, 2 equiv of concd HCl, rt, 24 h) resulted in the formation of the desired all-*syn*-substituted oxazine **4** in essentially quantitative yield and with at least a 94:6 diastereomeric ratio (by ^{1}H NMR). The relative and absolute stereochemistry of the newly created stereogenic center in the major diastereomer of **4** was determined as "*R*" by ^{1}H NMR NOE measurements that revealed a *syn*-relationship of the protons at C2, C5, and C6 of the oxazine ring. The high degree of asymmetric induction in the reduction step can be readily explained by adsorption of the substrate on the catalyst surface and subsequent hydrogenation of the double bond from the sterically less-hindered face of the molecule.

Protection of the secondary amine in **4** was achieved by treatment with Cbz−Cl (Et_3N, DMAP, CH_2Cl_2, rt) to obtain the desired *N*-Cbz-morpholine **5** in 96% yield (Scheme 2). Reduction of the ester group in **5** was achieved by using DIBALH (CH_2Cl_2, −78 °C) to obtain the corresponding aldehyde **6** in 85% yield. Conversion of aldehyde **6** to the *β*-alkoxy imine **7** was achieved in excellent yield by treatment with benzylamine in the presence of alumina.

Chelation-controlled allylation of imine **7** was studied by using allyl−metal reagents in the presence of various Lewis acids. Thus, treatment of **7** with allyltrimethylsilane or allyltributyltin in the presence of a Lewis acid ($TiCl_4$, $BF_3{\cdot}Et_2O$, or $AlCl_3$; −78 to 0 °C) resulted in the formation of the desired homoallylamine **8**, however, in low yields (30−45%) and with poor diastereoselectivity

SCHEME 3



(*dr* = 1.5−2:1). Fortunately, use of allylzinc bromide in the presence of anhydrous cerium chloride[11] (1.1 equiv) proved to be beneficial and resulted in the formation of **8** in essentially quantitative yield and with good diastereoselectivity (*dr* = 4.4:1, by ^{1}H NMR) as a nonseparable mixture of diastereomers (Scheme 3).

The stereoselectivity of the allylation reaction is reasonably assumed to be controlled by stereoelectronic as well as steric effects. Considering the six-membered transition state ***B*** (Scheme 3) generated because of the chelation of $CeCl_3$ with the *β*-alkoxy imine, we speculated the axial attack of the allylzinc reagent would generate the desired homoallylamine with *anti*-diastereoselectivity. The stereochemistry of the newly created stereogenic center in the major diatereomer of **8** was confirmed by its conversion into (+)-negamycin (**1**). It should be noted that the application of catalytic $CeCl_3$ in Zn-mediated allylations of chiral imines has been reported in the literature.[12]

As shown in Scheme 4, protection of the nitrogen atom in the homoallylamine **8** (*dr* = 4.4:1) was achieved by using Cbz−Cl (1 M NaOH, dioxane, 0 °C, 80%), furnishing the bis(Cbz) derivative **9** (*dr* = 4.4:1). Oxidative cleavage of the carbon−carbon double bond in **9** (O_3, MeOH, CH_2Cl_2, −78 °C; then Ph_3P) and purification by chromatography generated the aldehyde **10** in 73% yield and as a single diastereomer. Further oxidation of aldehyde **10** with PDC (DMF, rt) generated the desired carboxylic acid **11** in 97% yield.

Condensation of **11** with the PTSA salt of benzyl (1-methylhydrazino)acetate[6h] was performed by using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (Et_3N, HOBT, CH_2Cl_2, rt) to obtain the coupling product **12** in 80% yield. Hydrogenolysis of **12** (40 psi of H_2, 10% Pd/C, MeOH, H_2O, AcOH, 75 °C) produced the acetate salt of (+)-negamycin, which on purification by ion-exchange chromatography (Amberlite CG-50, NH_4^+ form) afforded (+)-negamycin (**1**)[6] in 75% yield ($[\alpha]_D^{25} = +1.9$ (*c* 1, H_2O); lit.[6h] $[\alpha]_D = +1.7$ (*c* 0.6, H_2O); lit.[6b] $[\alpha]_D^{20} = +2.3$ (*c* 4.07, H_2O)). The spectroscopic data for this substance matched those reported in the literature for (+)-negamycin.

Conclusion

In conclusion, we have demonstrated a concise, asymmetric, and stereocontrolled method for the synthesis of

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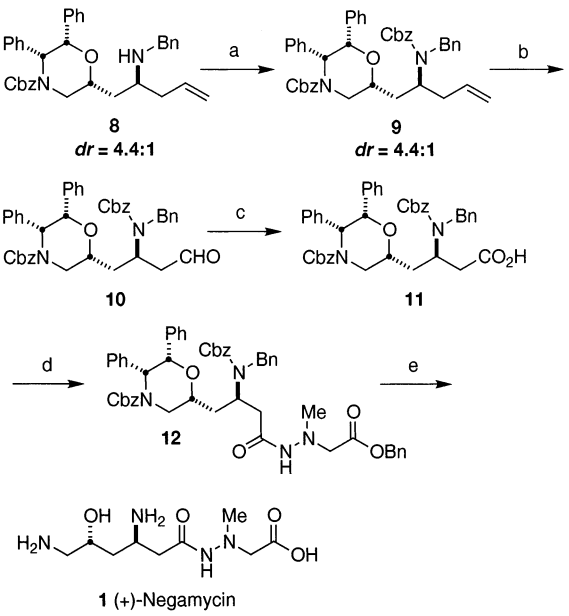
SCHEME 4[a]



[a] Reagents and conditions: (a) Cbz−Cl, 1 M NaOH, dioxane, 0−5 °C, 80%; (b) O_3, CH_2Cl_2, MeOH, −78 °C, 73%; (c) PDC, DMF, rt, 97%; (d) benzyl (1-methylhydrazino)acetate−PTSA salt, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, Et_3N, HOBT, CH_2Cl_2, rt, 80%; (e) 40 psi of H_2, 10% Pd/C (25 mol %), MeOH, H_2O, AcOH, 75 °C, 75%.

(+)-negamycin by using commercially available (5*R*,6*S*)-4-(benzyloxycarbonyl)-5,6-diphenyl-2,3,5,6-tetrahydro-4*H*-1,4-oxazin-2-one (**2**)[7] as a starting material. Current efforts are focused on extending the stabilized Wittig homologation on the oxazinones to other amino acid-derived natural products and alkaloids.

Experimental Section

General Methods. All reactions requiring anhydrous conditions were performed under a positive pressure of argon using oven-dried glassware (120 °C) that was cooled under argon. THF was distilled from sodium benzophenone ketyl, and dichloromethane was distilled from CaH_2. Column chromatography was performed on Merck silica gel Kieselgel 60 (230−400 mesh).

5*R*,6*S*-2-Methoxycarbonylmethyl-5,6-diphenyl-5,6-dihydro-[1,4]oxazine-4-carboxylic Acid Benzyl Ester (3). The mixture of **2** (2.23 g, 5.76 mmol, 1 equiv) and methyl (triphenylphosphoranylidene)acetate (4.80 g, 14.4 mmol, 2.5 equiv) in xylene (15 mL) was heated to 210 °C in a preheated oil bath for 2 h in a sealed tube. The mixture was then cooled to ambient temperature, and the solvent was removed under reduced pressure to obtain the crude product, which on purification by flash chromatography on silica gel (7:3 petroleum ether/ethyl acetate) furnished 2.55 g (100%) of **3** as a pale yellow gum. $[\alpha]_D^{25} = +202.8$ (*c* 2, $CHCl_3$); ^{1}H NMR (400 MHz, DMSO-d_6, 393 K) δ 7.29−6.95 (m, 15H), 6.70 (s, 1H), 5.34 (d, 1H, $J = 3.2$ Hz), 5.32 (d, 1H, $J = 3.2$ Hz), 5.17 (d, 1H, $J = 12.8$ Hz), 5.11 (d, 1H, $J = 12.8$ Hz), 3.68 (s, 3H), 3.39 (d, 1H, $J = 16.4$ Hz), 3.31 (d, 1H, $J = 16.4$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$, 300 K) (mixture of rotamers) δ 169.8, 151.3, 136.2, 136.1, 135.9, 135.6, 135.5, 134.1, 132.8, 128.2, 128.1, 128.0, 127.5, 127.4, 127.3, 127.1, 126.2, 126.0, 105.7, 104.9, 78.5, 78.3, 67.7, 67.3, 60.1, 58.9, 51.8, 37.5; IR ($CHCl_3$) 1743, 1705, 1605, 1586 cm^{-1}. HRMS (FAB+): calcd for $C_{27}H_{25}NO_5$ (*m*/*z*), 443.1732; found (*m*/*z*), 443.1729.

2*R*,5*R*,6*S*-2-(Methoxycarbonylmethyl)-5,6-diphenylmorpholine Hydrochloride (4). To a solution of **3** (2.77 g, 6.25 mmol, 1 equiv) in MeOH (140 mL) were added HCl (10 M, 1.25 mL, 12.5 mmol, 2 equiv) and $PdCl_2$ (0.22 g, 1.25 mmol, 0.2 equiv), and the mixture was stirred under 115 psi of H_2 and at ambient temperature for 24 h. The catalyst was removed by filtration through Celite, and the pad of Celite was washed with hot MeOH. The combined filtrates were concentrated under reduced pressure to obtain 2.17 g (99%) of crude **4** (*dr* = 94:6 by ^{1}H NMR) as a yellow amorphous powder which was pure by NMR. $[\alpha]_D^{25} = +49.7$ (*c* 1, MeOH); ^{1}H NMR (300 MHz, CD_3OD, 300 K) δ 7.63−7.60 (m, 2H), 7.34−7.09 (m, 8H), 5.38 (d, 1H, $J = 3.3$ Hz), 4.99 (d, 1H, $J = 3.3$ Hz), 4.63−4.54 (m, 1H), 3.77 (s, 3H), 3.43−3.29 (m, 2H), 2.95 (d, 2H, $J = 6.3$ Hz); minor diastereomer (visible peaks) δ 5.61 (d, $J = 3.6$ Hz), 3.69 (s); ^{13}C NMR (100 MHz, CD_3OD, 300 K) δ 171.9, 137.9, 132.8, 132.4, 130.7, 129.8, 129.3, 128.7, 126.5, 79.0, 72.7, 58.5, 52.7, 41.6, 38.6; IR (KBr) 3000−2500 (br), 1738, 1604, 1583 cm^{-1}. HRMS (FAB+): calcd for $C_{19}H_{22}NO_3$ (*m*/*z*), 312.1599; found (*m*/*z*), 312.1599.

2*R*,5*R*,6*S*-2-(Methoxycarbonylmethyl)-5,6-diphenylmorpholine-4-carboxylic Acid Benzyl Ester (5). To a solution of **4** (2 g, 5.76 mmol, 1 equiv) in anhydrous CH_2Cl_2 (30 mL) were added Et_3N (3.22 mL, 23.10 mmol, 4 equiv) and DMAP (0.071 g, 0.576 mmol, 0.1 equiv). To this stirred mixture was added dropwise Cbz−Cl (1.24 mL, 8.68 mmol, 1.5 equiv) over a period of 15 min, and the mixture was stirred at ambient temperature for 5.5 h. The reaction mixture was concentrated under reduced pressure and purified by flash chromatography on silica gel (8:2 petroleum ether/ethyl acetate) to furnish 2.46 g (96%) of **5** as a clear colorless gum. $[\alpha]_D^{25} = -62.8$ (*c* 1, $CHCl_3$); ^{1}H NMR (400 MHz, DMSO-d_6, 393 K) δ 7.37−7.06 (m, 15H), 5.38 (d, 1H, $J = 3.2$ Hz), 5.20 (d, 1H, $J = 12.8$ Hz), 5.14 (d, 1H, $J = 12.8$ Hz), 5.13 (d, 1H, $J = 3.2$ Hz), 4.27−4.20 (m, 1H), 4.06 (dd, 1H, $J = 3.2$, 13.2 Hz), 3.69 (s, 3H), 3.18 (dd, 1H, $J = 11.2$, 13.2 Hz), 2.85 (dd, 1H, $J = 5.6$, 15.6 Hz), 2.79 (dd, 1H, $J = 6.8$, 15.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, 300 K) (mixture of rotamers) δ 170.6, 170.5, 155.1, 155.0, 138.1, 138.0, 136.4, 136.3, 136.2, 130.1, 129.7, 128.4, 128.1, 128.0, 127.9, 127.8, 127.7, 127.3, 126.9, 125.6, 125.4, 79.9, 79.8, 73.4, 73.2, 67.6, 67.4, 57.7, 56.7, 51.9, 43.2, 42.9, 38.6, 38.4; IR ($CHCl_3$) 1740, 1699, 1604, 1585 cm^{-1}. HRMS (FAB+): calcd for $C_{27}H_{28}NO_5$ (*m*/*z*), 446.1967; found (*m*/*z*), 446.1971.

2*R*,5*R*,6*S*-2-(2-Oxoethyl)-5,6-diphenylmorpholine-4-carboxylic Acid Benzyl Ester (6). To a solution of **5** (1.9 g, 4.26 mmol, 1 equiv) in anhydrous CH_2Cl_2 (40 mL) was added at −78 °C DIBALH (1 M solution in toluene, 8.54 mL, 8.54 mmol, 2 equiv) dropwise over a period of 30 min. The reaction mixture was further stirred at −78 °C for an additional 2 h, after which it was quenched by adding 50 mL of water. The reaction mixture was filtered through a pad of Celite, dried (Na_2SO_4), and concentrated under reduced pressure to obtain the crude **6**, which on purification by flash chromatography on silica gel (85:15 petroleum ether/ethyl acetate) furnished 1.63 g (85%) of **6** as a white amorphous solid. $[\alpha]_D^{25} = -70.4$ (*c* 1.7, $CHCl_3$); ^{1}H NMR (300 MHz, DMSO-d_6, 393 K) δ 9.83 (t, 1H, $J = 1.5$ Hz), 7.35−7.06 (m, 15H), 5.38 (d, 1H, $J = 3.6$ Hz), 5.21−5.11 (m, 3H), 4.44−4.35 (m, 1H), 4.05 (dd, 1H, $J = 3.3$, 13.5 Hz), 3.15 (dd, 1H, $J = 11.4$, 13.5 Hz), 2.89−2.86 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$, 300 K) (mixture of rotamers) δ 198.9, 198.8, 154.7, 154.6, 137.6, 137.5, 136.0, 135.9, 129.7, 129.3, 128.2, 127.8, 127.6, 127.5, 127.1, 126.7, 125.2, 125.1, 79.7, 71.9, 71.7, 67.4, 67.3, 57.5, 56.6, 46.6, 46.5, 43.1, 42.9; IR ($CHCl_3$) 1725, 1698, 1604, 1585 cm^{-1}. HRMS (FAB+): calcd for $C_{26}H_{26}NO_4$ (*m*/*z*), 416.1861; found (*m*/*z*), 416.1870.

2*R*,5*R*,6*S*-2-(2-Benzyliminoethyl)-5,6-diphenylmorpholine-4-carboxylic Acid Benzyl Ester (7). To a solution of **6** (1.5 g, 3.61 mmol, 1 equiv) in anhydrous CH_2Cl_2 (15 mL) was added at 0 °C neutral Al_2O_3 (3 g), followed by dropwise addition of benzylamine (394 μL, 3.61 mmol, 1 equiv). The reaction mixture was further stirred at 0 °C for an additional 30 min, after which it was filtered through a pad of Celite and concentrated under reduced pressure to obtain 1.79 g (98%) of crude **7** as a white amorphous solid which was pure by NMR.

$[\alpha]_D^{25} = -41.3$ (*c* 1, $CHCl_3$); ^1H NMR (400 MHz, DMSO-d_6, 393 K) δ 8.01 (t, *J* = 4.8 Hz, 1H), 7.34−7.09 (m, 20H), 5.37 (d, 1H, *J* = 3.2 Hz), 5.18 (d, 1H, *J* = 12.4 Hz), 5.13 (d, 1H, *J* = 12.4 Hz), 5.10 (d, 1H, *J* = 3.2 Hz), 4.60 (s, 2H), 4.26−4.21 (m, 1H), 4.07 (dd, 1H, *J* = 3.2, 13.2 Hz), 3.14 (dd, 1H, *J* = 11.2, 13.2 Hz), 2.75 (t, 2H, *J* = 5.6 Hz); ^{13}C NMR (75 MHz, $CDCl_3$, 300 K) (mixture of rotamers) δ 162.0, 161.9, 155.1, 155.0, 138.7, 138.2, 138.1, 136.4, 136.2, 130.0, 129.7, 128.4, 128.0, 127.8, 127.2, 126.9, 125.4, 79.9, 79.8, 74.4, 67.6, 67.4, 67.3, 65.1, 57.9, 57.8, 56.9, 56.8, 43.4, 43.3, 39.8, 39.5; IR ($CHCl_3$) 1697, 1604, 1584 cm^{-1}. HRMS (FAB+): calcd for $C_{33}H_{33}N_2O_3$ (*m*/*z*), 505.2491; found (*m*/*z*), 505.2499.

2*R*,2′*S*,5*R*,6*S*-2-(2′-Benzylaminopent-4′-enyl)-5,6-diphenylmorpholine-4-carboxylic Acid Benzyl Ester (8). To a solution of **7** (1.82 g, 3.61 mmol, 1 equiv) in anhydrous THF (20 mL) was added at −40 °C a 1 M suspension of anhydrous $CeCl_3$ in THF (4 mL, 4 mmol, 1.1 equiv) dropwise over a period of 10 min. The mixture was stirred at −40 °C for 4 h. To the resulting milky suspension was added a solution of allylzinc bromide in THF (prepared by dropwise addition of allyl bromide (1.29 mL, 14.5 mmol, 4 equiv) to a vigorously stirred suspension of Zn (0.975 g, 14.5 mmol, 4 equiv) in anhydrous THF (15 mL) over a period of 30 min in a 25 °C water bath and by stirring the mixture for an additional 3 h) over a period of 15 min. The reaction mixture was further stirred at −40 °C for an additional 30 min, after which it was quenched by adding 50 mL of water and 50 mL of 2 M HCl. The mixture was warmed to rt and diluted with ethyl acetate (200 mL). The organic layer was separated and washed with water (50 mL), 1 M NaOH (50 mL), water (50 mL), and brine (50 mL). The organic layer was dried (Na_2SO_4) and concentrated to obtain 1.9 g (96%) of crude **8** (*dr* = 4.4:1) as a clear colorless gum which was pure by NMR. $[\alpha]_D^{25} = -26.2$ (*c* 1, $CHCl_3$); ^1H NMR (300 MHz, DMSO-d_6, 393 K) δ 8.08−8.00 (m, 1H), 7.33−7.05 (m, 20H), 5.98−5.85 (m, 1H), 5.36 (d, 1H, *J* = 3.3 Hz), 5.18 (d, 1H, *J* = 12.6 Hz), 5.12 (d, 1H, *J* = 12.6 Hz), 5.10−5.02 (m, 2H), 4.53 (br, 1H), 4.10−4.01 (m, 1H), 3.96 (dd, 1H, *J* = 3.6, 13.2 Hz), 3.84 (d, 1H, *J* = 13.2 Hz), 3.06 (d, 1H, *J* = 13.2 Hz), 3.96 (dd, 1H, *J* = 11.1, 13.2 Hz), 3.04−2.94 (m, 1H), 2.40−2.24 (m, 2H), 1.91−1.82 (m, 1H), 1.73−1.64 (m, 1H); IR ($CHCl_3$) 2925, 1697, 1639, 1604, 1585 cm^{-1}. HRMS (FAB+): calcd for $C_{36}H_{39}N_2O_3$ (*m*/*z*), 547.2960; found (*m*/*z*), 547.2965.

2*R*,2′*S*,5*R*,6*S*-2-(2′-(Benzylbenzyloxycarbonylamino)-pent-4′-enyl)-5,6-diphenylmorpholine-4-carboxylic Acid Benzyl Ester (9). To a solution of **8** (1.9 g, 3.47 mmol, 1 equiv) in dioxane (50 mL) was added 1 M NaOH (10 mL, 10 mmol), and the mixture was cooled in an ice−water bath (0−5 °C). To this mixture was added Cbz−Cl (991 μL, 6.94 mmol, 2 equiv) dropwise, and the reaction mixture was further stirred at the same temperature for 12 h, after which it was diluted with ethyl acetate (100 mL). The organic layer was separated, washed with water (50 mL) followed by brine (50 mL), dried (Na_2SO_4), and concentrated to afford the crude product, which on purfication by flash chromatography on silica gel (8:2 petroleum ether/ethyl acetate) furnished 1.9 g (80%) of **9** as a clear colorless gum. $[\alpha]_D^{25} = -13.2$ (*c* 1, $CHCl_3$); ^1H NMR (400 MHz, DMSO-d_6, 393 K) δ 7.34−7.03 (m, 25H), 5.68−5.58 (m, 1H), 5.27 (d, 1H, *J* = 3.6 Hz), 5.14−4.87 (m, 6H), 4.81 (d, 1H, *J* = 3.6 Hz), 4.48 (d, 1H, *J* = 16.0 Hz), 4.31 (d, 1H, *J* = 16.0 Hz), 4.22−4.12 (m, 1H), 3.64−3.54 (m, 2H), 2.88−2.82 (m, 1H), 2.43−2.30 (m, 2H), 1.99−1.90 (m, 1H), 1.82−1.75 (m, 1H); IR ($CHCl_3$) 1697, 1604, 1584 cm^{-1}. HRMS (FAB+): calcd for $C_{44}H_{45}N_2O_5$ (*m*/*z*), 681.3328; found (*m*/*z*), 681.3341.

2*R*,2′*R*,5*R*,6*S*-2-(2′-(Benzylbenzyloxycarbonylamino)-4′-oxobutyl)-5,6-diphenylmorpholine-4-carboxylic Acid Benzyl Ester (10). To a solution of **9** (1.4 g, 2 mmol, 1 equiv) in anhydrous MeOH (40 mL) and anhydrous CH_2Cl_2 (20 mL) was bubbled O_3 at −78 °C until the solution turned blue. At this point, Ar gas was bubbled through the solution for 5 min to remove excess O_3. Ph_3P (0.682 g, 2.6 mmol, 1.3 equiv) was added, and the mixture was warmed to and stirred at ambient temperature overnight. CH_2Cl_2 was distilled off at 1 atm, and the solution was refluxed in residual methanol for 30 min. Removal of solvent under reduced pressure afforded crude **10**, which on purification by chromatography on silica gel (8:2 petroleum ether/ethyl acetate) furnished 1.03 g (73%) of **10** as a white amorphous solid. $[\alpha]_D^{25} = -17.0$ (*c* 1.4, $CHCl_3$); ^1H NMR (300 MHz, DMSO-d_6, 393 K) δ 9.51 (t, 1H, *J* = 1.8 Hz), 7.37−7.07 (m, 25H), 5.30 (d, 1H, *J* = 3.6 Hz), 5.17 (d, 1H, *J* = 12.6 Hz), 5.15 (d, 1H, *J* = 12.3 Hz), 5.12 (d, 1H, *J* = 12.6 Hz), 5.06 (d, 1H, *J* = 12.3 Hz), 4.86 (d, 1H, *J* = 3.6 Hz), 4.65−4.57 (m, 1H), 4.54 (d, 1H, *J* = 15.6 Hz), 4.40 (d, 1H, *J* = 15.6), 3.71−3.60 (m, 2H), 2.91 (dd, 1H, *J* = 11.1, 13.2 Hz), 2.81 (dd, 2H, *J* = 1.8, 6.9 Hz), 2.11−2.04 (m, 1H), 1.94−1.84 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$, 300 K) (mixture of rotamers) δ 199.9, 199.4, 156.6, 155.1, 154.9, 154.8, 138.1, 138.0, 137.7, 137.5, 136.3, 136.1, 129.8, 129.5, 128.3, 128.0, 127.9, 127.7, 127.6, 127.5, 127.4, 127.1, 126.9, 126.8, 125.5, 125.3, 79.4, 73.7, 73.3, 72.9, 67.4, 67.3, 67.0, 57.9, 57.1, 52.2, 50.8, 48.9, 48.6, 47.8, 43.5, 43.3, 36.8, 35.8; IR ($CHCl_3$) 1721, 1696, 1604, 1584 cm^{-1}. HRMS (FAB+): calcd for $C_{43}H_{43}N_2O_6$ (*m*/*z*), 683.3121; found (*m*/*z*), 683.3134.

2*R*,2′*R*,5*R*,6*S*-2-(2′-(Benzylbenzyloxycarbonylamino)-3′-carboxypropyl)-5,6-diphenylmorpholine-4-carboxylic Acid Benzyl Ester (11). To the solution of **10** (0.85 g, 1.24 mmol, 1 equiv) in anhydrous DMF (4 mL) was added PDC (1.632 g, 4.34 mmol, 3.5 equiv), and the mixture was stirred at ambient temperature for 12 h. The solvent was removed under reduced pressure, and the residue was purified by flash chromatography on silica gel (ethyl acetate) to furnish 0.85 g (97%) of **11** as a white amorphous solid. $[\alpha]_D^{25} = -28.0$ (*c* 1, $CHCl_3$); ^1H NMR (400 MHz, DMSO-d_6, 373 K) δ 11.80 (br, 1H), 7.39−7.07 (m, 25H), 5.31 (br, 1H), 5.18 (d, 1H, *J* = 12.8 Hz), 5.15 (d, 1H, *J* = 12.8 Hz), 5.13 (d, 1H, *J* = 12.8 Hz), 5.07 (d, 1H, *J* = 12.8 Hz), 4.81 (d, 1H, *J* = 3.6 Hz), 4.56−4.10 (m, 1H), 4.52 (d, 1H, *J* = 16.0 Hz), 4.45 (d, 1H, *J* = 16.0 Hz), 3.66−3.57 (m, 2H), 2.88 (dd, 1H, *J* = 11.2, 12.8 Hz), 2.67 (d, 2H, *J* = 7.2 Hz), 2.10−1.98 (m, 1H), 1.93−1.86 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$, 300 K) (mixture of rotamers) δ 176.7, 176.1, 156.8, 155.0, 138.2, 138.1, 137.6, 136.3, 136.1, 129.9, 129.5, 128.3, 128.0, 127.9, 127.7, 127.6, 127.3, 127.1, 126.8, 126.7, 125.5, 125.3, 79.3, 73.7, 73.3, 73.0, 67.5, 67.4, 67.0, 57.9, 57.0, 53.4, 52.9, 51.5, 43.6, 43.3, 39.1, 38.0, 36.8, 36.5, 35.8; IR ($CHCl_3$) 1729, 1698, 1604, 1585 cm^{-1}. HRMS (FAB+): calcd for $C_{43}H_{43}N_2O_7$ (*m*/*z*), 699.3070; found (*m*/*z*), 699.3050.

Synthesis of 12. To a mixture of **11** (0.85 g, 1.21 mmol, 1 equiv), benzyl (1-methylhydrazino)acetate−PTSA salt (0.886 g, 2.42 mmol, 2 equiv), and HOBT (0.327 g, 2.42 mmol, 2 equiv) in anhydrous CH_2Cl_2 (10 mL) was added Et_3N (1.7 mL, 12.1 mmol, 10 equiv) which was followed by dropwise addition of a solution of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (0.464 g, 2.42 mmol, 2 equiv) in anhydrous CH_2Cl_2 (20 mL). The reaction mixture was stirred at ambient temperature for 12 h. Solvent was removed under reduced pressure, and the residue was dissolved in ethyl acetate (200 mL) and washed with 1 M HCl (5 × 50 mL), water (1 × 50 mL), 1 M NaOH (3 × 50 mL), water (1 × 50 mL), and brine (1 × 50 mL). The organic layer was dried (Na_2SO_4) and concentrated to obtain the crude **12**, which on purification by flash chromatography on silica gel (1:1 petroleum ether/ethyl acetate) furnished 0.857 g (80%) of **12** as a white amorphous solid. $[\alpha]_D^{25} = -23.6$ (*c* 1, MeOH); ^1H NMR (400 MHz, DMSO-d_6, 393 K) δ 8.69 (br, 1H), 7.38−7.06 (m, 30H), 5.31 (d, 1H, *J* = 3.2 Hz), 5.18 (d, 1H, *J* = 12.4 Hz), 5.15 (d, 1H, *J* = 12.4 Hz), 5.14 (d, 1H, *J* = 12.4 Hz), 5.07 (d, 1H, *J* = 12.4 Hz), 5.14 (s, 2H), 4.78 (d, 1H, *J* = 3.2 Hz), 4.49 (d, 1H, *J* = 12.0), 4.45 (d, 1H, *J* = 12.0), 4.54−4.42 (m, 1H), 3.66−3.56 (m, 4H), 2.86 (t, 1H, *J* = 11.6 Hz), 2.62 (br, 5H), 2.05−1.99 (m, 1H), 1.93−1.82 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$, 300 K) (mixture of rotamers) δ 174.1, 173.6, 170.1, 169.4, 168.7, 168.3, 156.8, 156.6, 155.1, 154.9, 138.2, 138.1, 137.6, 136.4, 136.3, 136.2, 134.9, 129.8, 129.5, 128.4, 128.3, 128.2, 128.1, 127.9, 127.8, 127.6, 127.5, 127.2, 127.0, 126.6, 126.5, 125.5, 125.4, 125.3, 79.2, 79.0, 73.8, 73.6, 73.2, 72.9, 72.7, 67.3, 67.2, 66.9, 66.7,

66.4, 58.4, 57.8, 57.7, 57.4, 57.0, 56.9, 54.5, 53.5, 52.7, 44.7, 43.6, 43.5, 43.3, 43.2, 39.8, 38.5, 36.8, 36.4, 35.7; IR ($CHCl_3$) 3325, 1737, 1694, 1605, 1585 cm^{-1}. HRMS (FAB+): calcd for $C_{53}H_{55}N_4O_8$ (*m*/*z*), 875.4019; found (*m*/*z*), 875.4002.

(+)-Negamycin (1). To a solution of **12** (0.2 g, 0.22 mmol, 1 equiv) in MeOH (24 mL) were added AcOH (1.2 g, 20 mmol) and water (6 mL). To this clear solution was added in portions 10% Pd/C (0.06 g, 0.056 mmol, 0.25 equiv), and the mixture was hydrogenated under 40 psi of H_2 and at 75 °C for 4−4.5 h. The reaction mixture was filtered through a pad of Celite and concentrated under reduced pressure. The residue was dissolved in water and purified on Amberlite CG-50 resin ($NH4^+$ form), eluting with 1.5% aq NH_4OH. The eluents were concentrated under reduced pressure to furnish 0.043 g (75%) of **1** as a white powder. $[\alpha]_D^{25} = +1.9$ (*c* 1, H_2O); ^1H NMR (300 MHz, D_2O, 300 K) δ 3.86−3.78 (m, 1H), 3.30−3.18 (m, 1H), 3.21 (s, 2H), 2.87 (dd, 1H, J = 3.3, 12.9 Hz), 2.70 (dd, 1H, J = 9.0, 12.9 Hz), 2.45 (s, 3H), 2.20 (d, 2H, J = 6.3 Hz), 1.46−1.39 (m, 2H); ^{13}C NMR (100 MHz, D_2O, 300 K) δ 177.2, 170.9, 65.6, 60.9, 45.2, 45.0, 43.9, 41.1, 39.5; IR (KBr) 3500−2500, 1662, 1602, 1450, 1401, 1315, 1131 cm^{-1}. HRMS (FAB+): calcd for $C_9H_{21}N_4O_4$ (*m*/*z*), 249.1562; found (*m*/*z*), 249.1565.

Acknowledgment. This work was supported by the National Science Foundation (Grant CHE 9731947). We thank Prof. Louis S. Hegedus (Department of Chemistry, Colorado State University) for providing the ^1H and ^{13}C NMR spectra of authentic negamycin.

Supporting Information Available: ^1H and/or ^{13}C spectra of all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

JO025636I