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Total synthesis of (+)-negamycin and its 5-*epi*-derivative

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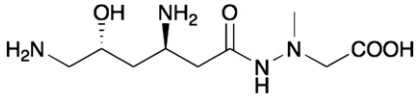
ABSTRACT

(+)-Negamycin was prepared in 13 steps in an overall yield of 31% from commercially available ethyl (*R*)-(+)-4-chloro-3-hydroxybutanoate. The key step in the reaction sequence was a highly stereoselective asymmetric Michael addition of chiral amine (−)-**21** [(1*S*,2*R*)-(−)-2-methoxybornyl-10-benzylamine] into the α,β-unsaturated carbonyl moiety of key intermediate **8**, thus establishing the second chiral center in (+)-negamycin. 5-*epi*-Negamycin was also prepared in a similar fashion.

1. Introduction

The unusual antibiotic (+)-negamycin (**1**) containing a hydrazine peptide bond was first isolated by Umezawa et al. in 1970 from culture filtrates of three strains closely related to *Streptomyces purpeofuscus*.[1,2] (+)-Negamycin was found to exhibit inhibitory activity toward multi drug-resistant Gram-positive and Gram-negative bacteria,[1] and it was also found to be a specific inhibitor of protein biosynthesis with miscoding activity.[3] In 2003 Matsuda et al. reported that (+)-negamycin was found to restore dystrophin expression in skeletal and cardiac muscles of *mdx* mice, an animal model of Duchenne muscular dystrophy (DMD).[4] The first total synthesis of (+)-negamycin was reported in 1972 and this work confirmed the assigned structure for the natural product.[5] Since this early work, a number of total syntheses have been reported of racemic negamycin[6] and (+)-negamycin.[7] There are also several reports of formal total synthesis of natural product **1**.[8] Our own interest regarding this compound was triggered by the latest findings by Matsuda and co-workers and an efficient total synthesis of (+)-negamycin was recently communicated (Fig 1).[9] Herein we report on our second approach toward this intriguing natural product starting from commercially available ethyl (*R*)-(+)-4-chloro-3-hydroxybutanoate **6**. We also report our synthesis of 5-*epi*-negamycin **25**, which only has been synthesized by one group previously.[7i]



(+)-Negamycin (**1**)

Figure 1. Chemical structure of (+)-negamycin.

2. Results and discussion

In order to secure an expedient synthesis of (+)-negamycin **1**, two synthetic routes outlined in the retrosynthetic analysis were embarked upon (Scheme 1). The analysis suggested that natural product **1** could be prepared from hydrazine **2**[7i] and amino acid derivative **3** or **7** in route A and B, respectively. Compound **3** in route A could in its place come from substrate **4** via an new asymmetric Michael addition reaction[10b] followed by hydrolysis of the ester functionality. This stereoselective amination method was recently developed by Node and co-workers[11,12] using chiral methoxybornyl-10-benzylamine as a chiral amine source, and the bulky *tert*-butyl ester was a crucial functional moiety to induce high stereoselectivity. Key intermediate **4** could be derived from aldehyde **5**, which could be prepared from relatively cheap commercially available chiral ester **6** via several functional group interconversions. The azide functionality within substrates **5**, **4** and **3** was envisioned functioning as a 'protected' amine group, which could be unmasked at the very last step of the synthesis, i.e., the final deprotection step. Realizing that the azide group could function as a masked amine meant that the synthetic pathway toward (+)-negamycin could be reduced by potentially two steps (protection of the amino functionality and deprotection). If successful,

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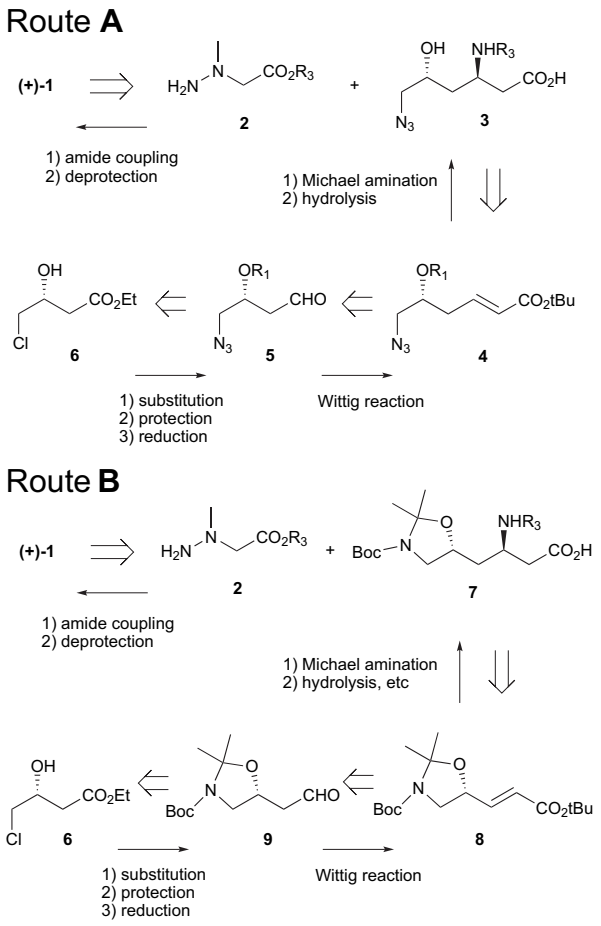
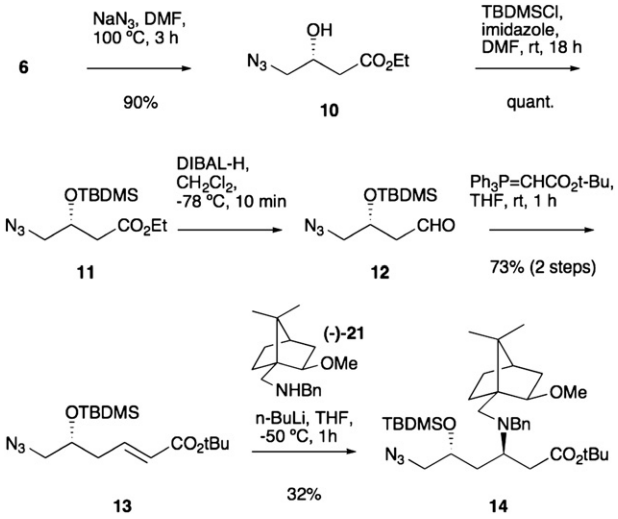


Scheme 1. Retrosynthetic analysis of (+)-negamycin **1**.

the strategy outlined below with its eight steps would facilitate a short total synthesis of (+)-negamycin. In contrast, in route B the terminal amino functionality was planned to be constructed in the early stage of the synthesis, while we envisaged installing the amino functionality by using a similar asymmetric Michael addition reaction on the key intermediate olefin **8**. Route B required more steps, including protection and deprotection of the terminal amino group. However, the unstable and potentially explosive nature of the azide group in Route A can mostly be avoided.



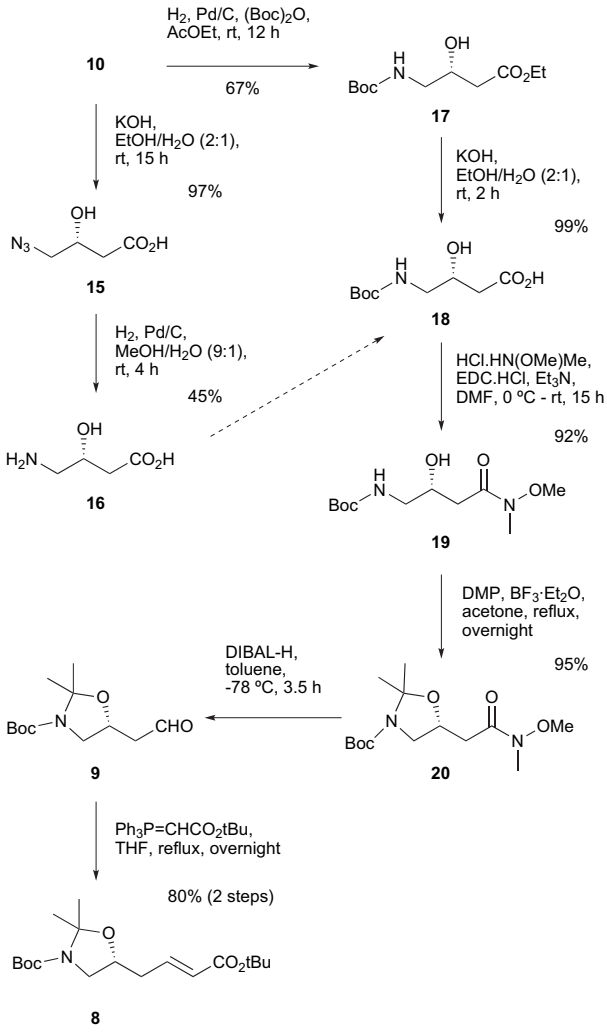
Scheme 2. Synthesis of Michael adduct **14** in Route A.

The synthesis of (+)-negamycin **1** started with the conversion of the commercially available ethyl (*R*)-(+)-4-chloro-3-hydroxybutanoate **6** over three steps to aldehyde **12** as depicted in Scheme 2, by using a method developed by Hirai et al. for the synthesis of the opposite enantiomer of aldehyde **12**.[13] The chloride functionality within ester **6** was first converted to the corresponding azide **10** upon treatment with sodium azide in DMF at 100 °C. Attempts to convert the unprotected alcohol **10** directly to the corresponding aldehyde only resulted in formation of trace amounts of the desired product together with a small amount of the corresponding diol. The free hydroxyl functionality was therefore first protected as the TBDMS ether **11**, which was next treated with DIBAL-H in CH_2Cl_2 at −78 °C, thus forming the desired aldehyde **12**.

Due to the slightly unstable nature of aldehyde **12**, it was used directly in the Wittig reaction without purification. Namely, aldehyde **12** was treated with (*tert*-butoxycarbonylmethylene)-triphenylphosphorane in THF for 1 h to afford the desired ester **13** in 73% yield over 2 steps after purification by flash chromatography on silica. Proton NMR analysis of substrate **13** revealed that the sole product of this reaction was the compound with *E*-geometry as evident from the large coupling constants for the vinylic protons (16 Hz). However, the asymmetric Michael addition to compound **14** proceeded in low yield (32%). This might be due to the instability of substrate **13**. From TLC analysis of the crude product **13**, it was clear that this compound was the only product formed during the Wittig reaction from aldehyde **12**. However, compound **13** was sensitive to the slightly acidic silica gel, which resulted in partly decomposition during flash chromatography. Ester **13** was also sensitive toward storage and decomposed to a complex mixture of compounds rapidly. The unstable nature of ester **13** hampered the progress of the synthesis via Route A. Hence, the alternative Route B was considered to be more suitable.

For the strategy outlined in Route B, the use of (*R*)-(+)-4-amino-3-hydroxybutyric acid **16** [nicknamed (*R*)-(−)-GABOB], a readily prepared intermediate, was viewed as a good starting point (Scheme 3). (*R*)-(−)-GABOB has previously been prepared via a number of routes using various starting materials.[14] To this end, ester **10** was hydrolyzed followed by reduction of the azide functionality within compound **15** to (*R*)-(−)-GABOB **16** using a literature procedure reported by Larchevêque and Henrot.[14c] However, the yield for the reduction was relatively low (45%) and a different order of events was found to be more efficient. Reduction of azide **10** under an atmosphere of hydrogen over Pd/C (10%) in the presence of Boc_2O gave the corresponding Boc protected (*R*)-(−)-GABOB derivative **17** in 68% yield. Hydrolysis of the ester group upon treatment with KOH in ethanol/water 2:1 gave the corresponding acid **18** in an excellent yield (99%). Acid **18** was then converted to Weinreb amide **19** followed by protection of the free hydroxyl group and amide nitrogen as an oxazolidinone, thus forming amide **20** in 95% yield. Weinreb amide **20** was then converted to aldehyde **9**, which was treated directly with (*tert*-butoxycarbonylmethylene)triphenylphosphorane in THF under reflux overnight. This gave, after purification by flash chromatography on silica, key intermediate **8** in 80% yield over the two steps. With compound **8** in hand we were nicely set-up for the asymmetric Michael addition,[10] which we envisioned being executed by utilizing the methodology developed by Node and co-workers Scheme 4.[11]

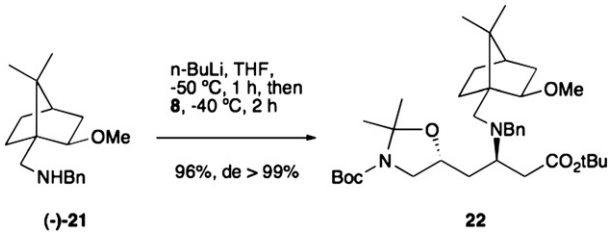
As reported previously by us, chiral auxiliary (−)-**21**[9,12] was first converted to the corresponding lithium salt at −50 °C. Slow addition of intermediate **8** to the resulting reaction mixture resulted in smooth conversion to the desired product **22**, which was isolated in 96% yield based on recovered starting material (17% of intermediate **8** was recovered from the reaction mixture and recycled) (80% yield in Ref. 9). Key intermediate **22** was formed as a single diastereomer (de >99%) as judged by ^1H NMR analysis. Interestingly the



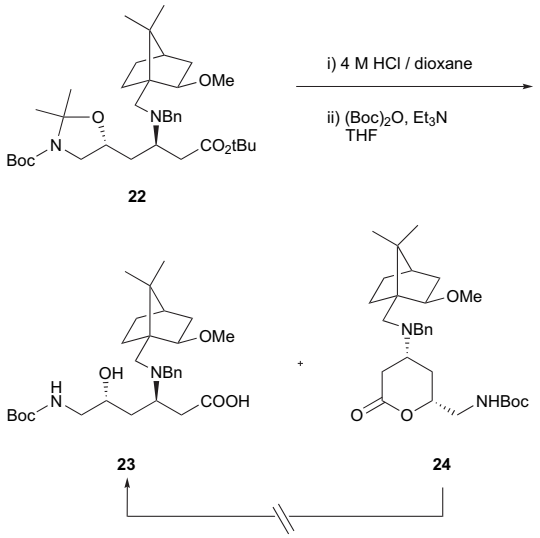
Scheme 3. Synthesis of key intermediate **8** in Route B.

stereochemistry at the C-5 position did not influence the stereoselectivity in our asymmetric Michael addition. The total yield of Michael adduct **22** from starting material **6** was 41%.

With the two chiral centers embodied in (+)-negamycin (**1**) securely in place, focus now shifted toward the latter half of the synthesis. The question was whether we should hydrolyze the ester functionality first in order to install the hydrazine moiety prior to cleave the chiral auxiliary or inversely. To examine these possibilities, compound **22** was treated with 4 M HCl in dioxane, resulting in hydrolysis of the *t*-butyl ester functionality as well as removal of the oxazolidinone and Boc protection groups (Scheme 5). The Boc group was then reinstalled by treating the crude product mixture with Boc_2O and Et_3N in THF. This gave acid **23** in a low yield (25%) together with the undesired lactone **24** (75% yield). Attempts to

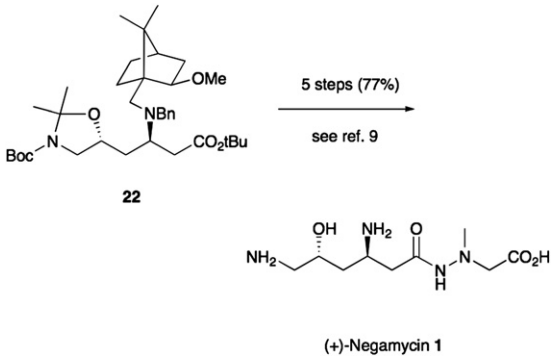


Scheme 4. Asymmetric Michael addition using chiral methoxybornyl-10-benzylamine as a chiral amine source.



Scheme 5. Formation of stable *δ*-lactone **24**.

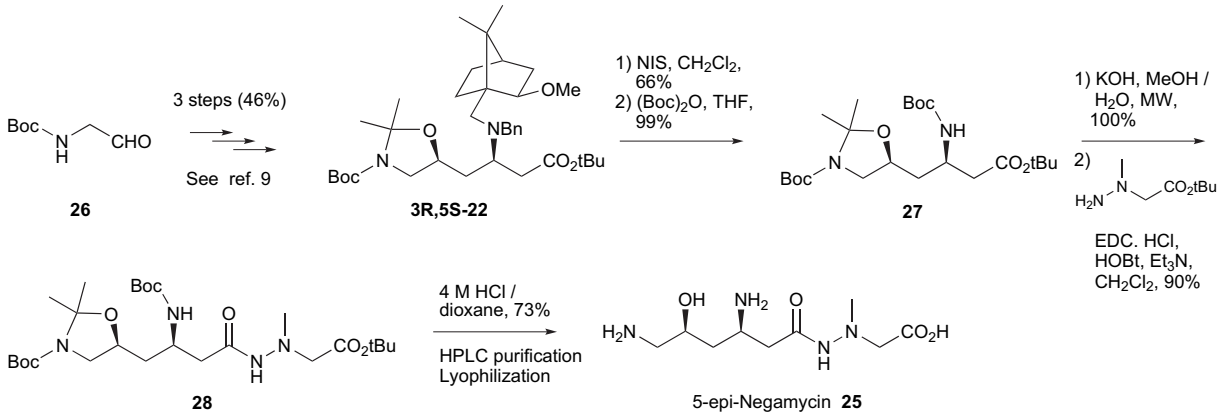
convert lactone **24** to the desired product **23** failed. The alternate procedure, namely removing the chiral auxiliary upon treatment with NIS in dichloromethane,[12] was successful and resulted in the formation of (+)-negamycin **1** over five steps using chemistry recently disclosed by us (Scheme 6).[9]



Scheme 6. Synthesis of (+)-negamycin **1**.

(+)-Negamycin **1** was by these means prepared in an overall yield of 31% over 13 steps. The spectroscopic and physical data obtained for the target compound was in full accord with the data reported in the literature. Although the overall yield is lower than our most efficient route to this compound, which uses an expensive Grubbs' Catalyst (Second Generation) for the key cross-metathesis reaction.[9] The yield obtained for compound **1** compares favorably with most previous synthesis of (+)-negamycin.[5,7]

For the purpose of preparing a sample of 5-*epi*-negamycin **25** for biological testing, the opposite stereoisomer at the 5 position viz. compound **3*R*,5*S*-22** was synthesized according to the previously reported method.[9] Namely, the synthesis was started from achiral *N*-Boc-2-aminoacetaldehyde, followed by an asymmetric allylboration using (−)-*B*-allyldiisopinocampheylborane [(−)-Ipc_2B(allyl)] with the opposite stereochemistry, cross-metathesis reaction using Grubbs second-generation [Ru-**II**] catalyst and asymmetric Michael addition (data not shown). The obtained compound **3*R*,5*S*-22** was subjected to the same sequence of reactions as just described for (+)-negamycin in order to give 5-*epi*-negamycin **25** in 41% yield as the TFA salt from substrate **3*R*,5*S*-22** (Scheme 7).



Scheme 7. Synthesis of 5-*epi*-negamycin.

3. Conclusion

The synthetic strategy outlined here resulted in the preparation of (+)-negamycin in 13 steps with an overall yield of 31% starting from commercially available chiral starting material. By utilizing the same synthetic strategy a sample of 5-*epi*-negamycin was prepared. This synthetic strategy together with our previously reported approach[9] is able to facilitate the preparation of a range of analogues of the natural product for biological evaluation. Work toward this end is currently ongoing in our laboratories.

4. Experimental

4.1. General procedures

Melting points were measured on a Yanagimoto micro hot-stage apparatus and are uncorrected. Proton (^{1}H) and carbon (^{13}C) NMR spectra were recorded on either a JEOL JNM-AL300 spectrometer operating at 300 MHz for proton and 75 MHz for carbon, or a Varian UNITY INOVA 400NB spectrometer operating at 400 MHz for proton and 100 MHz for carbon. Chemical shifts were recorded as δ values in parts per million (ppm) downfield from tetramethylsilane (TMS). Low- and high-resolution mass spectra (CI and EI) were recorded on a JEOL JMS-GCmate. Optical rotations were measured with a Horiba High-speed Accurate Polarimeter SEPA-300 at the sodium-D line (589 nm) at the concentrations (*c*, g 100 mL^{-1}). The measurements were carried out between 24–28 °C in a cell with path length (*l*) of 0.5 dm. Specific rotations $[\alpha]_D$ are given in 10^{-1} deg cm^2 g^{-1}. Preparative HPLC was performed using a C18 reverse-phase column (25,020 mm; YMC-Pack ODS-AM) with the binary solvent system.

4.1.1. Ethyl (R)-4-azido-3-hydroxybutanoate ***10***. Sodium azide (2.77 g, 42.6 mmol) was added to a stirred solution of ethyl (*R*)-(+)-4-chloro-3-hydroxybutanoate **6** (3.50 g, 21.0 mmol) in DMF (55 mL) at room temperature. The resulting reaction mixture was then heated at 100 °C for 3 h before being cooled to room temperature and filtered. The remaining salt was washed with ether (3×10 mL) and filtered. The combined organic fractions were concentrated under reduced pressure and the resulting yellow oil was subjected to flash column chromatography (silica, *n*-hexane/EtOAc=8:2→7:3 gradient eluent). Concentration of the relevant fractions (R_f 0.3 in *n*-hexane/EtOAc=7:3) under reduced pressure gave the title azide **10**[13] (3.28 g, 90%) as a light-yellow oil. $[\alpha]_D^{28}$+7.5° (*c* 3.65, MeOH), lit.[13] $[\alpha]_D^{20}$+7.4° (*c* 4.05, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.19 (q, *J*=7.2 Hz, 2H), 4.12 (partly obscured m, 1H), 3.35 (ddd, *J*=12, 6.0 and 4.6 Hz, 2H), 3.31 (s, 1H), 2.54 (dd, *J*=2.9 and 0.7 Hz, 2H), 1.29 (t, *J*=7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.0, 67.3, 61.0, 55.5, 38.4, 14.1; HRMS (CI+) calcd for $C_6H_{12}N_3O_3$ (M^++H) 174.0878, found 174.0875.

4.1.2. Ethyl (3R)-4-azido-3-tert-butyldimethylsilyloxybutanoate ***11***. Imidazole (47.9 mg, 0.70 mmol) was added to a stirred solution of alcohol **10** (52.5 mg, 0.303 mmol) and *tert*-butyldimethylsilyl chloride (88.1 mg, 0.584 mmol) in DMF (2.5 mL) at room temperature. The resulting reaction mixture was then stirred at room temperature for 18 h before being diluted with EtOAc (15 mL) and washed with water/brine (5×10 mL of a 1:1 solution) and brine (1×10 mL) before being dried ($MgSO_4$). Filtration and concentration under reduced pressure gave a light-yellow oil, which was subjected to flash column chromatography (silica, *n*-hexane→*n*-hexane/EtOAc=9:1 gradient eluent). Concentration of the relevant fractions (R_f 0.4 in *n*-hexane/EtOAc=9:1) gave the title compound **11** (86.9 mg, quant.) as a clear colorless oil. $[\alpha]_D^{25}$+2.2° (*c* 0.33, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.23 (dt, *J*=6.2 and 5.2 Hz, 1H), 4.13 (dd, *J*=2.4 and 7.1 Hz, 2H), 3.37 (dd, *J*=4.3 and 13 Hz, 1H), 3.24 (dd, *J*=5.3 and 13 Hz, 1H), 2.54 (dd, *J*=3.8, and 6.2 Hz, 2H), 1.27 (t, *J*=7.1 Hz, 3H), 0.89 (s, 9H), 0.13 (s, 3H), 0.09 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.8, 68.7, 60.6, 56.4, 40.1, 25.6, 17.9, 14.1,−4.2, −5.7; MS (CI+) *m*/*z* 288 (M^++H, 14%), 272 (12), 245 (32), 231 (48), 230 (100), 156 (8), 115 (22), 73 (60); HRMS (CI+) calcd for $C_{12}H_{26}N_3O_3Si$ (M^++H) 288.1743, found 288.1739.

4.1.3. tert-Butyl (5R)-6-azido-5-tert-butyldimethylsilyloxy-(2E)-hexenoate ***13***. DIBAL-H (293 μL of a 1.02 M solution in hexane, 0.299 mmol) was added dropwise to a solution of ester **11** (66.0 mg, 0.230 mmol) in dry CH_2Cl_2 (7.7 mL) at −78 °C. The reaction mixture was stirred at −78 °C for 10 min, before the reaction mixture was quenched with water (10 mL) and stirred vigorously for 5 min. The mixture solution was filtered through a pad of Celite® with $CHCl_3$ and washed with H_2O, brine. The combined organic layer was dried over Na_2SO_4. Filtration and concentration under reduced pressure gave as an unclear oil. The product was used directly in the next reaction without further purification. (*tert*-Butoxycarbonylmethylene)triphenylphosphorane (113 mg, 0.299 mmol) was added to a stirred solution of aldehyde **12** in THF (2.3 mL) at room temperature. The reaction mixture was then stirred at room temperature for 1 h. The solvent was removed under reduced pressure and extracted with EtOAc (15 mL) and washed with satd $NaHCO_3$ aq (2×10 mL), brine (1×10 mL) before being dried over Na_2SO_4. Filtration and concentration under reduced pressure gave a yellow oil, which was subjected to flash column chromatography (silica, *n*-hexane/EtOAc=9:1). Concentration of the relevant fractions (R_f 0.8 in *n*-hexane/EtOAc=2:1) gave the title compound **13** (57.2 mg, 73% over 2 steps) as

a colorless oil. $[\alpha]_D^{26}$ −21.7° (*c* 1.00, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 6.77 (dt, *J*=7.7 and 16 Hz, 1H), 5.79 (dt, *J*=1.4 and 16 Hz, 1H), 3.89 (dt, *J*=10 and 5.4 Hz, 1H), 3.27 (dd, *J*=4.4 and 13 Hz, 1H), 3.16 (dd, *J*=5.8 and 13 Hz, 1H), 2.43–2.36 (m, 2H), 1.48 (s, 9H), 0.91 (s, 9H), 0.12 (s, 3H), 0.09 (s, 3H); HRMS (ES+) calcd for $C_{16}H_{32}N_3O_3Si$ (M^++H) 342.2213, found 342.2207.

4.1.4. tert-Butyl (5R,3R)-6-azido-5-tert-butyldimethylsilyloxy-3-(N-benzyl-N-(2-methoxy-7,7-dimethylbicyclo[2.2.1]heptan-1-ylmethyl)-amino)hexanoate ***14***. A 1.55 M solution of *n*-butyllithium in hexane (290 μL) was added dropwise to a solution of (−)-**21** (122 mg, 0.448 mmol) in dry THF (1.5 mL) and the mixture was stirred at −50 °C. After stirring for 30 min, a solution of **13** (76.6 mg, 0.224 mmol) in anhydrous THF (1.2 mL) was added dropwise to the reaction mixture, which was stirred at −40 °C for another 1 h. The reaction was quenched by adding a satd $NaHCO_3$ aq and the mixture was extracted with ether. The organic layer was washed with brine and dried over Na_2SO_4. After the solvent was concentrated under reduced pressure. The residue was purified by flash column chromatography (*n*-hexane/EtOAc=12:1) to afford desired product **14** (43.8 mg, 32%) as a colorless oil. ^1H NMR (300 MHz, $CDCl_3$) δ 7.39–7.28 (m, 4H), 7.25–7.18 (m, 1H), 3.85 (d, *J*=14 Hz, 1H), 3.77–3.69 (m, 1H), 3.39–3.34 (m, 1H), 3.33–3.30 (m, 1H), 3.28 (t, *J*=3.0 Hz, 1H), 3.13 (s, 3H), 3.18–2.92 (m, 2H, including 1H at δ 3.05, dd, *J*=13 and 6.2 Hz), 2.72 (d, *J*=14 Hz, 1H), 2.70 (dd, *J*=15 and 3.2 Hz, 1H), 2.21 (d, *J*=14 Hz, 1H), 2.05 (dd, *J*=15 and 9.5 Hz, 1H), 1.92–1.72 (m, 2H), 1.69–1.48 (m, 5H), 1.43 (s, 9H), 1.32–1.21 (m, 1H), 1.06–0.97 (m, 1H), 0.92 (s, 3H), 0.85 (s, 9H), 0.76 (s, 3H), 0.04, (s, 3H), −0.06 (s, 3H); HRMS (ES+) calcd for $C_{34}H_{59}N_4O_4Si$ (M^++H) 615.4306, found 615.4320.

4.1.5. (R)-4-Azido-3-hydroxybutanoic acid ***15***[14c]. Potassium hydroxide (1.72 g, 30.7 mmol) was added to a stirred solution of azide **10** (1.77 g, 10.2 mmol) in water (15 mL) and ethanol (30 mL) at room temperature. The reaction mixture was then stirred at room temperature for 15 h before ethanol was evaporated under reduced pressure. The resulting residue was extracted with ether (1×10 mL) and the water phase was acidified to pH 1 by addition of 3 M HCl. Water was then removed under reduced pressure and the residue thus obtained was dissolved in EtOAc and dried over $MgSO_4$. Filtration and concentration in vacuo gave the title acid **15**[14c] (1.44 g, 97%) as a yellow oil. $[\alpha]_D^{25}$ +2.7° (*c* 6.78, MeOH), lit.[14c] $[\alpha]_D^{20}$ +3.02° (*c* 2.55, MeOH); ^1H NMR (300 MHz, $CDCl_3$) δ 6.80–5.80 (br s, 2H), 4.27–4.20 (m, 1H), 3.42 (dd, *J*=4.4 and 13 Hz, 1H), 3.37 (dd, *J*=6.1 and 13 Hz, 1H), 2.63 (d, *J*=3.5 Hz, 1H), 2.61 (d, *J*=1.1 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.7, 67.1, 55.5, 38.2; HRMS (ES+) calcd for $C_4H_7N_3O_3Na$ (M^++Na) 168.0385, found 168.0372.

4.1.6. (R)-4-Amino-3-hydroxybutanoic acid [(−)-GABOB] ***16***[14c]. A suspension of acid **15** (1.41 g, 9.69 mmol) and Pd/C (10%) (104 mg) in methanol (29 mL) and water (3.2 mL) was stirred under an atmosphere of hydrogen at room temperature for 4 h. The reaction mixture was then filtered through a pad of Celite® and the filtrate was concentrated under reduced pressure. The resulting viscous yellow oil was dissolved in a small amount of water and dilution with absolute alcohol to give the title product as a brown solid. Recrystallization from water/methanol gave the title acid **16**[14c] (522 mg, 45%) as a white solid. Mp 214–215 °C, lit.[14c] Mp 212 °C; $[\alpha]_D^{25}$ −16.1° (*c* 0.69, H_2O), lit.[14c] $[\alpha]_D^{20}$ −20° (*c* 2.37, H_2O); ^1H NMR (400 MHz, D_2O) δ 4.21 (ddt, *J*=3.2, 6.6 and 9.4 Hz, 1H), 3.17 (dd, *J*=3.2 and 13 Hz, 1H), 2.95 (dd, *J*=9.4 and 13 Hz, 1H), 2.44 (d, *J*=6.6 Hz, 2H); MS (CI+) *m*/*z* 120 (M^++H, 100%), 103 (18), 102 (46), 84 (66), 57 (62); HRMS (CI+) calcd for $C_4H_{10}NO_3$ (M^++H) 120.0661, found 120.0657.

4.1.7. Ethyl 4-(N-tert-butyloxycarbonyl)amino-(R)-3-hydroxybutanoate ***17***. Di-*tert*-butyl dicarbonate (1.51 g, 6.93 mmol) and Pd/C (10%) (100 mg) were added to a stirred solution of alcohol **10** (1.00 g, 5.78 mmol) in EtOAc (60 mL) at room temperature. The resulting reaction mixture was subjected to three cycles of vacuum followed by flush with H_2 before being stirred vigorously under an atmosphere of H_2 for 12 h. The reaction mixture was then filtered through a pad of Celite® and washed with EtOAc (3×10 mL). The resulting filtrate was concentrated under reduced pressure and the resulting residue was subjected to flash column chromatography (silica, *n*-hexane/EtOAc=2:1). Concentration of the relevant fractions (R_f 0.3) gave the desired compound **17** (0.95 g, 67%) as a light-yellow oil. $[\alpha]_D^{25}$ +6.6° (*c* 1.06, $CHCl_3$); lit.[7j] $[\alpha]_D^{20}$ +6.6° (c 1.08, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 4.96 (br s, 1H), 4.18 (q, *J*=7.1 Hz, 2H), 4.14–4.06 (m, 1H), 3.46 (br s, 1H), 3.38–3.28 (m, 1H), 3.12 (ddd, *J*=5.7, 6.8 and 12 Hz, 1H), 2.49 (dd, *J*=2.4 and 5.6 Hz, 2H), 1.48 (s, 9H), 1.28 (t, *J*=7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.6, 156.5, 79.6, 67.7, 60.9, 45.5, 38.6, 28.3, 14.1; HRMS (CI+) calcd for $C_{11}H_{22}NO_5$ (M^++H) 248.1498, found 248.1504.

4.1.8. (R)-4-(tert-Butyloxycarbonyl)amino-3-hydroxybutanoic acid ***18***. KOH (1.36 g, 24.2 mmol) was added to a stirred solution of alcohol **17** (2.00 g, 8.00 mmol) in EtOH (24.5 mL) and water (12.3 mL) at room temperature. The resulting reaction mixture was stirred at room temperature for 2 h before EtOH was removed under reduced pressure. The resulting aqueous phase was acidified to pH 1 by addition of 10% citric acid aq and extracted with EtOAc (3×20 mL). The combined organic fraction was washed with brine (2×20 mL) before being dried (Na_2SO_4). The crude residue obtained after filtration and concentration under reduced pressure was subjected to flash column chromatography (silica, $CHCl_3$/MeOH=10:1). Concentration of the relevant fractions (R_f 0.4 in $CHCl_3$/MeOH=5:1) gave acid **18** (1.78 g, 99%) as a white solid. Mp 94–95 °C; $[\alpha]_D^{26}$ +9.7° (*c* 1.09, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 6.12 (br s, 1H), 5.18 (br s, 1H), 4.16–4.11 (m, 1H), 3.39–3.28 (m, 1H), 3.16 (ddd, *J*=1.3, 6.0 and 6.6 Hz, 1H), 2.53 (dd, *J*=5.3 and 2.2 Hz, 2H), 1.45 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.8, 157.0, 80.2, 67.8, 45.1, 38.5, 28.3; HRMS (CI+) calcd for $C_9H_{18}NO_5$ (M^++H) 220.1185, found 220.1184.

4.1.9. (R)-4-(tert-Butyloxycarbonyl)amino-3-hydroxy-N-methoxy-N-methylbutanamide ***19***. Triethylamine (2.00 mL, 14.6 mmol) was added dropwise to a solution of acid **18** (2.19 g, 13.3 mmol) and *N*,*O*-dimethylhydroxylamine hydrochloride (1.42 g, 14.6 mmol) in DMF (70 mL) maintained at 0 °C. The resulting reaction mixture was then stirred for 0.1 h at 0 °C before EDC·HCl (3.06 g, 16.0 mmol) was added. The reaction mixture was then allowed to heat to room temperature and stirred for 15 h before the solvent was removed under reduced pressure. The resulting residue was diluted with EtOAc (70 mL) and washed consecutively with 10% citric acid aq (1×20 mL), satd $NaHCO_3$ aq (1×20 mL), and brine (1×20 mL) before being dried over Na_2SO_4. Filtration and concentration under reduced pressure gave a light-yellow oil, which was purified by flash column chromatography (silica, $CHCl_3$/MeOH=10:1). Evaporation of the relevant fractions (R_f 0.6 in $CHCl_3$/MeOH=5:1) gave the desired compound **19** (3.21 g, 92%) as a colorless oil. $[\alpha]_D^{27}$ +27.8° (*c* 1.20, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 5.07 (br s, 1H), 4.16 (br s, 1H), 4.14–4.08 (m, 1H), 3.69 (s, 3H), 3.40–3.32 (m, 1H), 3.19 (s, 3H), 3.18–3.11 (m, 1H), 2.68 (d, *J*=17 Hz, 1H), 2.52 (dd, *J*=9.2 and 17 Hz, 1H), 1.45 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.2, 156.4, 79.4, 67.6, 61.3, 45.5, 35.3, 31.8, 28.3; HRMS (CI+) calcd for $C_{11}H_{23}N_2O_5$ (M^++H) 263.1607, found 263.1611.

4.1.10. 2-((R)-3-tert-Butyloxycarbonyl-2,2-dimethyloxazolidin-5-yl)-N-methoxy-N-methylacetamide ***20***. 2,2-Dimethoxypropane (6.02 mL, 49.1 mmol) and $BF_3 \cdot Et_2O$ (77.1 μL, 0.61 mmol) were added to a stirred solution of amide **19** (1.61 g, 6.14 mmol) in acetone (60 mL) at room temperature. The resulting reaction mixture was then heated at reflux overnight before being cooled to room temperature and poured into a solution of satd $NaHCO_3$ aq (30 mL). Acetone was then

removed under reduced pressure and the resulting residue was extracted with EtOAc (3×30 mL). The combined organic fraction was washed consecutively with satd $NaHCO_3$ aq (1×20 mL), and brine (1×20 mL) before being dried (Na_2SO_4). Filtration and concentration under reduced pressure gave a yellow oil, which was subjected to flash column chromatography (silica, $CHCl_3$/MeOH=15:1). Concentration of the relevant fractions (R_f 0.6 in *n*-hexane/EtOAc=1:1) gave desired compound **20** (1.73 g, 95%) as a light-yellow oil. $[\alpha]_D^{25}$ −18.4° (*c* 1.22, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.49 (br s, 1H), 3.87 (dd, *J*=5.6 and 10 Hz, 1H), 3.70 (s, 3H), 3.19 (s, 3H), 3.14–3.10 (m, 1H), 3.05–2.88 (m, 1H), 2.61 (dd, *J*=7.6 and 16 Hz, 1H), 1.58 (s, 3H), 1.51 (s, 3H), 1.46 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.0, 152.0, 93.1, 79.7, 70.2, 61.3, 51.2, 36.2, 31.9, 28.4, 26.7, 24.7; HRMS (CI+) calcd for $C_{14}H_{27}N_2O_5$ (M^++H) 303.1920, found 303.1925.

4.1.11. (E)-tert-Butyl 4-((R)-3-tert-butyloxycarbonyl-2,2-dimethyloxazolidin-5-yl)but-2-enoate ***8***. DIBAL-H (15.8 mL of a 0.99 M solution in toluene, 16.0 mmol) was added dropwise to a stirred solution of amide **20** (3.72 g, 12.3 mmol) in anhydrous toluene (123 mL) maintained under and argon atmosphere at −78 °C. The reaction mixture was then stirred for 3.5 h at −78 °C before being quenched by addition of ice-cold 1 M HCl. The aqueous phase was then extracted with EtOAc (3×20 mL) and the combined organic fraction was washed with satd $NaHCO_3$ aq (1×30 mL), and brine (1×30 mL) before being dried over Na_2SO_4. Filtration and concentration under reduced pressure gave 2-((*R*)-3-*tert*-butyloxycarbonyl-2,2-dimethyloxazolidin-5-yl)acetaldehyde **9**[7j] (2.95 g, 12.1 mmol) as a colorless oil. The product was used directly in the next reaction without further purification. *tert*-Buthoxycarbonylmethylenephenyl phosphorane (5.02 g, 13.3 mmol) was added to a stirred solution of aldehyde **9** (2.95 g, 12.1 mmol) in THF (121 mL) at room temperature. The reaction mixture was then heated at refluxed temperature for overnight before being cooled to rt. The reaction mixture was concentrated under reduced pressure and the resulting residue was diluted with EtOAc (80 mL) and washed consecutively with satd $NaHCO_3$ aq (1×30 mL), brine (1×30 mL) before being dried over Na_2SO_4. Filtration and concentration under reduced pressure gave a light-yellow oil, which was purified by flash column chromatography (silica, *n*-hexane/EtOAc=10:1). Concentration of the relevant fractions (R_f 0.4 in *n*-hexane/EtOAc=5:1) gave the desired compound **8** (1.95 g, 80% over 2 steps) as a colorless oil. $[\alpha]_D^{25}$ −26.2° (c 1.06, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.82 (ddd, *J*=14, 15 and 16 Hz, 1H), 5.85 (d, *J*=16 Hz, 1H), 4.19–4.14 (m, 1H), 3.73–3.65 (m, 1H), 3.13–3.04 (m, 1H), 2.55–2.42 (m, 2H), 1.57 (br s, 3H), 1.54 (br s, 3H), 1.48 (s, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.5, 152.2, 141.9, 125.8, 93.5, 80.4, 79.6, 72.1, 50.5, 35.6, 28.4, 28.1, 26.7, 24.8; HRMS (EI+) calcd for $C_{18}H_{31}NO_5$ (M^+) 341.2202, found 341.2200.

4.1.12. (R)-tert-Butyl-5-{(R)-3-(tert-butoxycarbonyl)-2-[N-benzyl-N-((2-methoxy-7,7-dimethylbicyclo[2,2,1]heptan-1-yl)methyl)amino]-propyl}-2,2-dimethyloxazolidine-3-carboxylate ***22***. The reaction was conducted as outlined in Ref. 9. $[\alpha]_D^{25}$ −14.0° (*c* 1.01, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29–7.26 (m, 4H), 7.22–7.18 (m, 1H), 4.31 (br s, 1H), 3.83 (d, *J*=14 Hz, 1H), 3.67–3.63, 3.57–3.53 (m, total 1H), 3.47 (dd, *J*=7.2 and 2.8 Hz, 1H), 3.32–3.27 (m, 1H), 3.25, 3.21 (s, total 1H), 3.18 (s, 3H), 3.01–2.93 (m, 2H), 2.81–2.70 (m, 2H), 2.18 (d, *J*=14 Hz, 1H), 2.12–2.03 (m, 1H), 1.79–1.73 (m, 2H), 1.65–1.26 (m, 12H), 1.47 (s, 9H), 1.43 (s, 9H), 0.91 (s, 3H), 0.63 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.4, 152.4, 140.7, 129.2, 128.0, 126.6, 93.2, 92.8, 85.0, 80.2, 79.8, 79.2, 70.7, 70.1, 54.8, 54.0, 52.6, 51.3, 50.9, 47.7, 45.5, 30.8, 28.5, 28.1, 27.4, 27.2, 26.4, 25.3, 24.4, 20.6, 20.4; HRMS (EI+) calcd for $C_{36}H_{58}N_2O_6$ (M+) 614.4294, found 614.4286.

4.1.13. (3R)-3-(Benzyl((2-methoxy-7,7-dimethylbicyclo[2.2.1]heptan-1-yl)methyl)amino)-6-(tert-butoxycarbonyl amino)-5-hydroxyhexanoic acid ***23***. Compound **22** (353 mg, 0.574 mmol) was treated with 4 M HCl/dioxane (5 mL) at 0 °C. The solution mixture was stirred at 60 °C for 1.5 h to completely remove three protective groups, i.e., Boc, dimethylacetal and *tert*-butyl ester. The solvent was removed under reduced pressure, and the resultant residue was used for the next reaction without further purification. The residue was dissolved in THF (5.7 mL), then Et_3N (160 μL, 1.15 mmol) and $(Boc)_2O$ (150 mg, 0.689 mmol) were added to this solution at 0 °C. The mixture was then stirred at room temperature for 3.5 h and the solvent was evaporated under reduced pressure. The resulting residue was extracted with EtOAc and the organic layer was washed with 10% citric acid aq, satd $NaHCO_3$ aq and brine, dried over Na_2SO_4. Filtration and concentration under reduced pressure gave a crude oil, which was purified by flash column chromatography ($CHCl_3$/MeOH=60:1) to give the desired compound **23** (77.3 mg, 25%) as a white solid. An undesired lactone **24** was also produced as a colorless oil in a higher yield (215 mg, 75%). Compound **23**; $[\alpha]_D^{25}$ −51.5° (*c* 1.00, $CHCl_3$); HRMS (ES+) calcd for $C_{29}H_{47}N_2O_6$ (M^++H) 519.3434, found 519.3397. Compound **24**; ^{1}H HMR (400 MHz, $CDCl_3$) δ 7.34–7.28 (m, 4H), 7.25–7.12 (m, 1H), 4.99 (br s, 1H), 4.15 (t, *J*=2.4 Hz, 1H), 3.80 (d, *J*=15 Hz, 1H), 3.61 (d, *J*=15 Hz, 1H), 3.48 (dd, *J*=7.3 and 3.2 Hz, 1H), 3.36 (dd, *J*=7.3 and 3.2 Hz, 1H), 3.23–3.10 (m, 5H, including 3H at δ 3.16, s), 2.81 (d, *J*=7.0 Hz, 1H), 2.78 (dd, *J*=18 and 9.2 Hz, 1H), 2.62 (dd, *J*=18 and 6.2 Hz, 1H), 2.20 (d, *J*=14 Hz, 1H), 1.99 (dt, *J*=14 and 2.4 Hz, 1H), 1.83–1.75 (m, 1H) 1.67–1.53 (m, 5H), 1.43 (s, 9H), 1.26–1.20 (m, 1H), 1.03–0.92 (m, 4H, including 3H at δ 0.96, s), 0.77 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.1, 160.0, 140.3, 128.4, 128.0, 126.9, 85.6, 79.7, 78.1, 55.8, 55.0, 52.9, 52.7, 47.5, 45.9, 45.3, 44.7, 37.5, 31.4, 31.2, 28.9, 28.4, 27.3, 20.8, 20.4; HRMS (ES+) calcd for $C_{29}H_{45}N_2O_5$ (M^++H) 501.3328, found 501.3346.

4.1.14. (+)-Negamycin (2-[(3R,5R)-3,6-diamino-5-hydroxyhexanoyl]-1-methylhydrazinoacetic acid) ***1***. (+)-Negamycin was prepared from compound **22** using our previously reported method.[9] The data for the compound was in full accord with the data reported previously.

4.1.15. (S)-tert-Butyl-5-{(R)-3-(tert-butoxycarbonyl)-2-[N-benzyl-N-((2-methoxy-7,7-dimethylbicyclo[2,2,1]-heptan-1-yl)methyl)amino]propyl}-2,2-dimethyloxazolidine-3-carboxylate ***3R,5S-22***. The reaction was conducted as outlined in Ref. 9. $[\alpha]_D^{25}$ +12.9° (*c* 1.10, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36–7.28 (m, 4H), 7.25–7.19 (m, 1H), 4.19 (br s, 1H), 3.91 (d, *J*=20 Hz, 1H), 3.44 (br s, 1H), 3.27 (d, *J*=14 Hz, 1H), 3.15 (s, 3H), 2.95–2.78 (m, 4H, including 1H at δ 2.72, dd, *J*=2.7 and 14 Hz), 2.62–2.55 (m, 1H), 2.30 (d, *J*=13 Hz, 1H), 2.05 (dd, *J*=10 and 14 Hz, 1H), 1.93 (t, *J*=11 Hz, 1H), 1.83 (d, *J*=11 Hz, 1H), 1.73–1.56 (m, 6H), 1.50–1.25 (m, 24H), 1.03 (t, *J*=9.4 Hz, 1H), 0.95 (s, 3H), 0.80 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.2, 152.0, 140.7, 130.0, 128.0, 127.9, 127.0, 92.8, 87.9, 85.6, 80.4, 79.0, 77.2, 71.6, 55.9, 54.9, 53.9, 52.9, 50.6, 47.8, 45.4, 45.0, 37.5, 36.5, 34.1, 31.3, 28.5, 28.2, 27.3, 24.2, 20.9, 20.5; HRMS (ES+) calcd for $C_{36}H_{59}N_2O_6$ (M^++H) 615.4373, found 615.4382.

4.1.16. (S)-tert-Butyl-5-{(R)-3-(tert-butoxycarbonyl)-2-tert-butoxycarbonylaminopropyl}-2,2-dimethyloxazolidine-3-carboxylate ***27***. The reaction was conducted as outlined in Ref. 9. $[\alpha]_D^{25}$ +26.7° (*c* 1.00, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.10 (br s, 1H), 4.18–4.07 (m, 1H), 3.98 (br s, 1H), 3.73 (br s, 1H), 3.04 (t, *J*=8.7 Hz, 1H), 2.55–2.42 (m, 2H), 1.91–1.74 (m, 2H), 1.53 (s, 6H), 1.50–1.34 (m, 32H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7, 155.3, 152.0, 146.8, 93.3, 85.2, 81.1, 79.3, 71.5, 51.1, 45.7, 40.7, 38.0, 28.5, 28.4, 27.5, 25.3; HRMS (ES+) calcd for $C_{23}H_{47}N_2O_7Na$ (M^++Na) 481.2914, found 481.2890.

4.1.17. (S)-tert-Butyl-(R)-5-[2-(tert-butoxycarbonylamino)-3-{(tert-butoxycarbonylmethyl)methylaminocarbamoyl}propyl]-2,2-dimethyloxazolidine-3-carboxylate ***28***. The reaction was conducted as

outlined in Ref. 9. $[\alpha]_D^{26}$ +15.5° (*c* 1.00, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 7.88, 7.36 (br s, total 1H), 5.55–5.30 (m, 1H), 4.16–3.92 (m, 2H), 3.71 (br s, 1H), 3.60–3.31 (δ 3.53, d, *J*=20 Hz and δ 3.35, d, *J*=18 Hz, total, 2H), 3.08 (t, *J*=9.6 Hz, 1H), 2.98–2.51 (m, 4H including 3H at δ 2.74, d, *J*=14 Hz), 2.38 (d, *J*=5.4 Hz, 1H), 2.04–1.78 (m, 2H), 1.60 (s, 3H), 1.50–1.32 (m, 30H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.4, 170.2, 169.6, 155.4, 151.9, 93.7, 82.3, 79.0, 71.5, 58.9, 50.9, 45.7, 43.9, 38.3, 36.9, 28.4, 28.3, 28.1, 26.8, 24.7; HRMS (ES+) calcd for $C_{26}H_{48}N_4O_8Na$ (M^++Na) 567.3370, found 567.3391.

4.1.18. 5-epi-Negamycin TFA salt (2-[(3R,5S)-3,6-diamino-5-hydroxyhexanoyl]-1-methylhydrazinoacetic acid) TFA salt ***25****.* 5-*epi*-Negamycin was prepared from compound **3*R*,5*S*-22** using the same method as we used to prepare (+)-negamycin.[9] Preparative HPLC was performed using a C18 reverse-phase column (250×20 mm; YMC-Pack ODS-AM) with a binary solvent system; a linear gradient of CH_3CN 0–5% in 0.1% aqueous TFA at a flow rate of 6 mL/min, detected at 222 nm. This gave after purification 5-*epi*-negamycin as the TFA salt in a 41% yield over the five steps. $[\alpha]_D^{25}$ −11.5° (*c* 0.55, H_2O), lit.[7i] $[\alpha]_D$ −9.4° (*c* 0.50, H_2O); ^1H NMR (400 MHz, D_2O) δ 4.14–4.08 (m, 1H), 3.92–3.85 (m, 1H), 3.67 (s, 2H), 3.20 (dd, *J*=3.2 and 13 Hz, 1H), 3.01 (dd, *J*=8.4 and 13 Hz, 1H), 2.79–2.69 (m, 4H, including 3H at δ 2.72, s), 2.62 (dd, *J*=7.2 and 16 Hz, 1H), 1.97–1.84 (m, 2H); ^{13}C NMR (100 MHz, D_2O) δ 173.7, 169.1, 65.3, 58.9, 46.9, 44.5, 44.2, 35.6, 35.0; HRMS (FAB+) calcd for $C_9H_{21}N_4O_4$ (M^++H) 249.1563, found 249.1559.

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Supplementary data

Supplementary data including ^1H and/or ^{13}C NMR charts of compounds **8**–**11**, **13**, **14**, **17**–**20**, **3*R*,5*S*-22**, **24**, **25**, **27** and **28**. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2009.10.097.

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